Gonad enhancement of green sea urchin (*Strongylocentrotus droebachiensis*): importance of feed and containment system

by

Julie Jacques

A thesis submitted to the
School of Graduate Studies
in partial fulfillment of the
requirements for the degree of
Master of Science in
Marine Biology

Department of Ocean Sciences

Memorial University of Newfoundland

September 2020

St. John's, Newfoundland, Canada

ABSTRACT

Wild sea urchins are harvested for their gonads (roe or uni) throughout coastal areas of the world. The high value of urchin gonads on global seafood markets along with increasing popularity and demand worldwide have led to the development of formulated-feed-based gonad enhancement programs since the early 1990s. Along the coast of eastern Canada, there is an abundance of green sea urchin, *Strongylocentrotus droebachiensis*, representing a largely untapped resource for gonad enhancement. To gain knowledge on the conditions and systems that optimize green sea urchin gonad production, two studies were performed with Newfoundland green sea urchins fed proprietary formulated feeds. In the first study, we carried out two gonad enhancement experiments with urchins fed in conical tanks at a water temperature of 1, 3, or 6°C. The first experiment lasted 4 wk with urchins collected before the spawning period, and the second lasted 8 wk with urchins collected during the spawning period. Feed consumption, feces production, and gonadosomatic index (GSI) all nearly doubled at 6 compared to 1°C, and urchins maintained good physiological condition and high gonad production, regardless of temporal proximity to spawning. However, the feed imparted a bitter gonad taste. In the second study, we carried out a 7-wk experiment in a tiered raceway system with urchins fed at three different stocking densities (2.5, 6.5, and 10.5 kg urchins m^{-2}), at a water temperature of 6°C. We also carried out concurrent trials with urchins fed kelp (*Laminaria digitata*), achieving a lower GSI than with the feed. Feed consumption was lowest in the most downstream raceway positions. Raceway position and urchin density influenced aggregation patterns, which reflected wild behaviours, however neither affected GSI. Regardless of different growing conditions and containment systems, in both studies urchins surpassed the GSI market target of ~10 to 15% in less than 7 wk, demonstrating the efficiency of formulated-feed-based gonad enhancement of Newfoundland green sea urchin.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Patrick Gagnon for all the support, guidance, and encouragement he provided throughout this project, and my committee members Dr. Harry Murray and Cyr Couturier for their support and constructive comments that helped improve this thesis. I am also grateful for Anne Provencher St-Pierre, Samantha Trueman, and David Bélanger for their continuous and generous support and assistance throughout all stages of this project, and Laura Teed, Sean Hacker Teper, Lindsee Clark, Cassidy Arnold, Aragorne Lomond, Laura Dawson, Alexandria Fudge, Sophia Wells, Laura Lilly, and Hailey Turnbull for their assistance with laboratory work and image analysis. I thank Neil Ollerhead for image analysis, Dr. David Schneider for support with experimental design, Danny Boyce and the JBARB staff for their help during laboratory work, and the field services team for diving assistance. Special thanks to our industry partners Brian Tsuyoshi Takeda (Urchinomics), Mark Sheppard (Green Seafoods Ltd), and Guy Vigneault and Peter Dinet (Pêcheries Shipek S.E.C.) for their time, support, and commitment. I also thank all of my family and friends for their constant support and encouragement throughout my thesis. This research was funded by Natural Sciences and Engineering Research Council (NSERC; Discovery Grant [RGPIN-2015-05343]; Engage Grants for universities [506750-16 and 517775-17]; and Engage Plus Grants [536872-18]), Canada Foundation for Innovation (CFI; Leaders Opportunity Funds [16940]), and Research & Development Corporation of Newfoundland and Labrador (RDC; Ignite R&D [5003.070.002]) grants to Patrick Gagnon. I was financially supported by the NSERC (CGS M) and FRQNT (B1) scholarships.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CO-AUTHORSHIP STATEMENT

The work described in the present thesis was conducted by Julie Jacques under the main supervision of Patrick Gagnon. David Bélanger and Samantha Trueman carried out the experimental trials described in Chapter II as part of contractual research assistantships and the project was designed, funded, and supervised by Patrick Gagnon. Julie Jacques was responsible for the design of the experiment and data collection and analysis in Chapter III, with assistance from Patrick Gagnon, David Schneider, and Neil Ollerhead. All data were analyzed, and chapters written, by Julie Jacques with intellectual and editorial input by Patrick Gagnon, Harry Murray, and Cyr Couturier. Publications in the primary literature resulting from the present thesis work will be co-authored by Julie Jacques, Patrick Gagnon, David Bélanger, and Samantha Trueman (Chapter II) and by Julie Jacques, Patrick Gagnon, Neil Ollerhead, and David Schneider (Chapter III).

CHAPTER I

General Introduction

In recent years, there has been a global increase in the popularity of sea urchins on the seafood market (Stefánsson et al. 2017). Despite this increase, global sea urchin supply has experienced a decline of ~40% since 1995, from ~120,000 to 75,000 tonnes (Stefánsson et al. 2017). Japan is the dominant consumer of sea urchin gonads (also called roe/uni; Figure 1.1A), consuming approximately 80-90% of the global sea urchin supply (Stefánsson et al. 2017, Sun and Chiang 2015). Other important global harvesters include Chile, Russia, the United States, and Canada, all main suppliers of live and processed sea urchins to Japan (Stefánsson et al. 2017, Sun and Chiang 2015).

Canada harvests ~4,000 to 6,000 tonnes of sea urchins per year, including green sea urchin (*Strongylocentrotus droebachiensis*; Figure 1.1B), making up 70% of harvest, and red sea urchin (*Mesocentrotus franciscanus*) (DFO 2018, FAO 2016, Pearce and Robinson 2010, Stefánsson et al. 2017). Canada is estimated to supply Japan with 460 tonnes of live or processed sea urchins per year, representing only 8% of the urchin products supplied to Japan (Explorations Unlimited Inc. 2006). On Japanese markets, one tray of urchin gonads (~0.1-0.4 kg $tray^{-1}$) can reach up to ¥12,000 (~$150 CAD) for high quality gonads, and ¥3,000-4,000 (~$30-50 CAD) for lower quality gonads (Explorations Unlimited Inc. 2006). The recent decline in global supply of sea urchins due to overharvesting suggests that the demand for high quality and fairly-priced urchin gonads on Asian markets is not met (Stefánsson et al. 2017), meaning that Canada could increase harvest to help reach this demand and better take advantage of this profitable market.

Green sea urchin is a keystone species in shallow rocky subtidal habitats in the northern hemisphere as it is a dominant consumer (Scheibling and Hatcher 2007). In eastern Canada, including Newfoundland, green sea urchin forms dense aggregations that destructively graze kelp

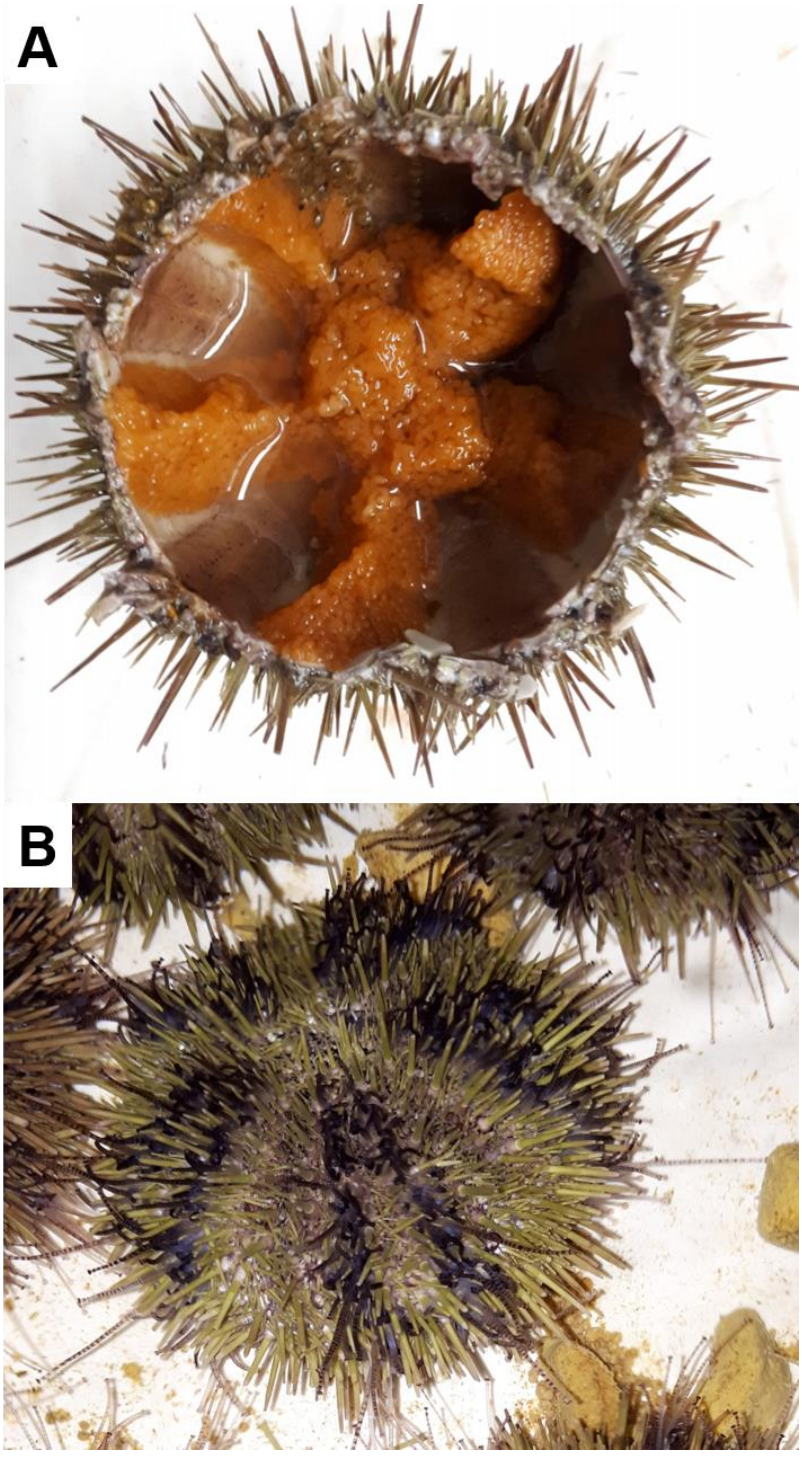

Figure 1.1 Newfoundland green sea urchin, *Strongylocentrotus droebachiensis*, (A) gonads and (B) feeding on a formulated feed pellet (yellowish C-shaped structures).

beds (Gagnon et al. 2004, Himmelman 1984, Scheibling et al. 1999, Steneck et al. 2002) and other seaweed assemblages (Blain and Gagnon 2014), leaving behind large areas of stripped seabed, termed urchin barrens (Blain and Gagnon 2014, Gagnon et al. 2004, Lawrence 1975, Steneck et al. 2002). The species therefore has a significant impact on the structure and dynamics of benthic communities (Scheibling and Hatcher 2007).

Sea urchin harvest in Canada mainly occurs in British Columbia, New Brunswick, and Newfoundland, however it is limited in Newfoundland since the fishery is relatively small and highly seasonal (Pisces Consulting Limited 2014, Stefánsson et al. 2017). For the harvest of Newfoundland green sea urchins, divers are largely targeting urchins at the lower edge of kelp beds since they often present larger gonads than urchins from barrens. Barrens urchins also have a lower mass and poorer quality, with a much lower market value (Frey and Gagnon 2016, Himmelman 1984, Himmelman and Steele 1971). Urchins are marketable if they achieve a gonad yield (gonad mass as a percentage of total body mass) of ~10 to 15% (Pisces Consulting Limited 2014). Additionally, urchins in Newfoundland typically only achieve marketable gonad mass in January to April close to their peak spawning period of March to May, limiting the timing of harvest (Himmelman 1978, Keats et al. 1984, Scheibling and Hatcher 2007).

These location and timing restrictions limit the successful harvest of marketable urchins, however the Newfoundland green sea urchin fishery could surpass current levels ($4 to 6.6 M; Pisces Consulting Limited 2014) by implementing gonad enhancement methods. In gonad enhancement, adult-sized urchins from wild populations are harvested, maintained in captivity, and fed natural or prepared diets with the aim of optimizing gonad yield, taste, colour, and texture (James et al. 2017, Robinson et al. 2002). In the study by Trueman (2019), marketable gonad yields were achieved in Newfoundland green sea urchins fed different kelp diets in land-based tanks over

12 to 34 weeks. This study was the first to demonstrate successful gonad enhancement in Newfoundland green sea urchins since the late 1990s (Trueman 2019). Although successful, further research must be performed on the various gonad enhancement techniques in order to determine optimal methods for the Newfoundland green sea urchin.

Although macroalgae are the typical diet of sea urchins (Scheibling et al. 1999), formulated feeds (Figure 1.1B) have been developed that allow urchins to reach market value more rapidly due to their higher nutritive value (Carrier et al. 2017, Pearce et al. 2004). Additionally, collecting kelp from the wild at multiple intervals to feed urchins throughout production poses many logistical issues due to seasonal fluctuations in kelp quantity and senescence (Carrier et al. 2017, Frey and Gagnon 2015, Himmelman 1984). However, a common issue with formulated feeds is the generally suboptimal gonad taste (often bitter and lacking the much-desired umami aftertaste), colour, and texture they impart (Pearce et al. 2002, 2003, 2004, Prato et al. 2018, Siikavuopio et al. 2007b). Therefore, new feed versions are constantly being developed to improve gonad yield and quality (Carrier et al. 2017, Pearce et al. 2004).

Gonad enhancement using formulated feeds to achieve marketable gonads has been successful in several studies with green sea urchin from Norway (Christiansen and Siikavuopio 2007, Siikavuopio et al. 2006, Siikavuopio et al. 2007a, Siikavuopio et al. 2007c, Siikavuopio et al. 2008), New Brunswick (Daggett et al. 2006), and Maine (Garrido and Barber 2001). Siikavuopio et al. (2008) studied feed intake and gonad yield at varying temperatures (between 4-14°C) and found that feed intake and gonad yield increased with increasing temperature. To study feeding over various seasons, Siikavuopio et al. (2007a) ran a feeding experiment spanning 848 days, and found that feed intake was higher in summer than in winter. Garrido and Barber (2001)

and Siikavuopio et al. (2006) tested the effects of temperature and season, and found that maximum gonad mass was achieved at a lower temperature in winter compared to summer.

Another important factor examined is stocking density since producers want to maximize the number of urchins in their system while minimizing the higher risk of mortality and cannibalism at higher densities (Jobling 1994). Christiansen and Siikavuopio (2007) found no significant differences in gonad yield, feed intake, and mortality among low stocking densities (less than 8 kg urchin m^{-2}). Siikavuopio et al. (2007c) also found that feed intake was consistent among higher densities (up to 16 kg urchin m^{-2}), but that gonad yield decreased and mortality increased with increasing density.

All these studies used land-based sea urchin containment systems, however gonad enhancement can also occur in sea-based cages preventing the need to pump water on land from the ocean (Daggett et al. 2006, Devin 2002). Land-based systems are beneficial in that the environmental conditions can be controlled and selected to maximize gonad growth (Daggett et al. 2006, Devin 2002). Maintaining proper conditions is important since poor water quality can negatively impact urchin health and gonad growth (Mortensen et al. 2012, Siikavuopio et al. 2004). In addition to maintaining water quality, ideal land-based containment systems must also maximize feed access, limit mortality, and facilitate removal of feces and debris to optimize urchin health and gonad yield (Daggett et al. 2006).

Urchins in land-based containment systems tend to form dense aggregations (Daggett et al. 2006, Devin 2002, Frey and Gagnon 2016), mirroring their behaviour in the wild of grouping together in dense aggregations (urchin fronts) to graze on kelp beds (Gagnon et al. 2004, Himmelman 1984, Scheibling et al. 1999, Steneck et al. 2002). Urchins also prefer positioning

themselves in corners and on vertical sides of tanks, complicating feed access since urchins must climb over one another to access the feed on the bottom of the tank (Daggett et al. 2006, Devin 2002, Siikavuopio et al. 2007c). Compared to deeper tanks, raceways (long, shallow troughs) improve feed access since the short vertical sides limit the available wall space, forcing urchins onto the horizontal bottom (Daggett et al. 2006, Devin 2002).

Another containment system design that is increasingly becoming more common involves the use of the long, shallow raceways, but stacking them so that water falls from the end of each raceway into the next one below (Daggett et al. 2006, Grosjean et al. 1998, Le Gall 1990). This system maximizes a facility's space use since it is built upward, taking up vertical space that is unused by tanks or unstacked raceways, and maximizes water use since the same water flows through each tier (Daggett et al. 2006). Since containment system design has been found to impact sea urchin growth, feed access, and mortality as well as feces and debris removal (Daggett et al. 2006, Devin 2002, James and Siikavuopio 2015), system effects must be considered in gonad enhancement studies.

Green sea urchin gonad enhancement has been successful at various temperatures, seasons, and densities and in various containment systems, however research is still needed on the Newfoundland green sea urchin since results may vary among geographical populations and the province is in need of science-based evidence that its green sea urchin resources are indeed amenable to gonad enhancement. The present thesis examines gonad enhancement in Newfoundland green sea urchin to gain knowledge on the conditions and systems that optimize gonad production. This thesis is the second of a planned series of studies exploring the potential for a sustainable green sea urchin industry in eastern Newfoundland, following the study by

Trueman (2019). Chapter II presents two 4- to 8-wk experimental studies on green sea urchins collected before and during the spawning period, and fed a formulated feed at different water temperatures in conical tanks. The effects of season and temperature on sea urchin feed consumption, feces production, physiological condition, gonad yield, and gonad taste were assessed. Chapter III presents a 7-wk experimental study on green sea urchins fed a diet of formulated feed or a diet of kelp at different stocking densities in a tiered raceway system to asses the effects of stocking density on sea urchin feed consumption, aggregation, and gonad yield. Chapter IV summarizes the main results of both studies, and discusses the implications for successful gonad enhancement of Newfoundland green sea urchin. Chapters II, III, and IV are written in a format compatible with the publication of research articles, which explains the repetition of information where appropriate, as well as the use of first-person plural pronoun ("we") and possessive determiner ("our") throughout.

CHAPTER II

Gonad enhancement in green sea urchin (*Strongylocentrotus droebachiensis*): importance of feed, water temperature, and temporal proximity to spawning

ABSTRACT

Wild sea urchins are harvested for their gonads (roe or uni) in many parts of the world, including along the eastern Canadian seaboard where green sea urchin, *Strongylocentrotus droebachiensis*, abounds and represents a largely untapped resource. The potentially high monetary return and the ever-increasing worldwide demand for green sea urchin gonad have contributed to the growth of formulated-feed-based echiniculture since the early 1990s. We carried out two gonad enhancement experiments with Newfoundland green sea urchins fed with a proprietary formulated feed. Both experiments (1) were carried out in a commercially available, low-flow containment system; (2) utilized urchins taken from barren grounds to ensure minimum gonad content to begin with; (3) differed in duration [4 or 8 wk] and timing of urchin collection from the field [just before or within the spawning period]; and (4) exposed urchins to one of three relatively low water temperatures [1, 3, or 6°C]. We showed that the feed represents a viable option to enhance gonads from ~6% to within the gonadosomatic index (GSI) market target of ~10 to 15% in only ~4 to 6 wk or ~50% less time than with a diet of purely kelp. Water temperature exerts a considerable influence on feed consumption, feces production, and gonad yield, which all nearly double at 6 compared to 1°C. Temporal proximity to spawning does not appreciably impact the urchin's overall physiological condition (as approximated with righting time), nor does it represent an obstacle to urchin gonad production, with virtually the same outcomes in unripe and ripe urchins. The only shortcoming of the feed is the bitter gonad taste it imparts, which increases with increasing seawater temperature and feed consumption. Nevertheless, a major advantage of the feed, containment system, and stable thermal/light environments used in the present study, is the ability to maintain high gonad production, while avoiding spawning, even at a time of the year when natural gonad growth is nearly arrested and urchins in the wild are largely spawning.

2.1 INTRODUCTION

Aquaculture is a vital component of global food supply (Boyd et al. 2019), accounting in 2018 for 46% of the global fish and shellfish production with an estimated 82M tonnes worth US$250 billion (FAO 2020). Aquaculture enables a far better production control than fisheries, substantially reducing the risks of overfishing, providing conservation of natural stocks, especially when carried from larval to adult stages (FAO 2020, FAO 2018, Robinson 2004, Siikavuopio et al. 2007c). Today's aquaculture industry largely focuses on finfish (carp, tilapia, and salmon), red seaweeds, crustaceans (shrimp, crawfish, and crab), and molluscs (mussels, oysters, and clams), with a smaller, yet growing demand for echinoderms, including sea cucumbers and sea urchins (FAO 2020, FAO 2018, Stefánsson et al. 2017). Sea urchins sold on the global market are predominantly sourced from fisheries, with less than 0.01% produced in aquaculture settings (James et al. 2016, Stefánsson et al. 2017).

Wild sea urchins are harvested for their gonads (roe or uni) in many parts of the world, including along the eastern Canadian seaboard where green sea urchin, *Strongylocentrotus droebachiensis*, abounds, particularly in Newfoundland (Andrew et al. 2002, Frey and Gagnon 2015, Gagnon et al. 2004, Keesing and Hall 1998, Pisces Consulting Limited 2014, Stefánsson et al. 2017). A relatively small green sea urchin fishery exists in Newfoundland, though its success and growth is limited due to variable gonad yield and quality, logistical challenges of collecting sea urchins when acceptable gonad yield and favourable sea conditions coincide, and the only government-approved method of collection being by hand, which is labor-intensive and time-consuming (Pisces Consulting Limited 2014). Depending on quality, the market price of green sea urchin gonads ranges from US$6 to $200 kg^{-1} (Robinson et al. 2002, Shpigel et al. 2005, Unuma 2002, Whitaker et al. 1997). With densities of up to ~300 individuals m^{-2}, *S. droebachiensis* is a

dominant component of rocky coastal habitats in Newfoundland (Blain and Gagnon 2014, Frey and Gagnon 2015, 2016, Gagnon et al. 2004, Himmelman 1984), representing a largely untapped resource.

The potentially high monetary return and the ever-increasing demand for green sea urchin gonad, particularly in Japanese markets (Explorations Unlimited Inc. 2006, Stefánsson et al. 2017, Sun and Chiang 2015), have contributed to echiniculture (sea urchin aquaculture) growth since the early 1990s (Pisces Consulting Limited 2014, Robinson et al. 2002). In gonad enhancement echiniculture, adult-sized urchins from wild populations are harvested, maintained in captivity, and fed natural or prepared diets with the aim of optimizing gonad yield, taste, colour, and texture (James et al. 2017, Robinson et al. 2002). Trueman (2019) recently examined the potential for green sea urchin echiniculture in Newfoundland, with three locally abundant kelp species (a combination of *Alaria esculenta* and *Laminaria digitata, L. digitata* alone, and *Agarum clathratum* alone) as feed options for gonad enhancement. *L. digitata* alone enhanced gonads to within the gonadosomatic index (GSI) market target of ~10 to 15% (Pisces Consulting Limited 2014) in 12 wk, whereas taste panelists ranked gonads of urchins fed the kelp combination or *L. digitata* alone as predominantly sweet, and those of urchins fed with *A. clathratum* alone, very bitter (Trueman 2019). Although promising, these diets proved somewhat challenging to provide, particularly during winter, because of the necessity to collect kelp from natural habitats and maintain, in land-based facilities, a constant supply of kelp to feed urchins *ad libitum*. The urchin containment systems used (flow through tanks with no evacuation system necessitating frequent manual removal of feces and debris) also was operationally limiting (Trueman 2019).

A variety of formulated feeds have been developed with the aim of increasing the mass of urchin gonads in less time than would be needed with entirely natural diets (e.g., raw kelp

material), ultimately limiting production costs (Azad et al. 2011, Carrier et al. 2017, Pearce et al. 2002). However, a common issue with formulated feeds is the generally suboptimal gonad taste (often bitter and lacking the much-desired umami aftertaste), colour, and texture they impart (Pearce et al. 2002, 2003, 2004, Prato et al. 2018, Siikavuopio et al. 2007b). Siikavuopio et al. (2006) and Siikavuopio et al. (2008) showed that formulated feed intake and gonad growth in green sea urchins generally increase with water temperature between 4 and 14°C. A corollary hypothesis, therefore, is that any particular gonad taste imparted by a given formulated feed should accentuate with water temperature since more feed is consumed (and in principle metabolically assimilated) in warmer water.

In the wild, green sea urchins spawn predominantly in the spring, typically from March to May (Himmelman 1978, Keats et al. 1984, Scheibling and Hatcher 2007). Following the spring spawning, gonad tissue (nutritive phagocytes, which are specialized cells that store nutrients; Himmelman 1978) grows at an accelerating rate until autumn, when primary oocyte proliferation and vitellogenesis/spermatogenesis begin (Scheibling and Hatcher 2007). Gonad growth continues during winter, though at a decelerating rate as ova and sperm mature and are stored until spawned (Scheibling and Hatcher 2007). Feeding in green sea urchin is linked with the reproductive cycle, with an increase during and after the spring spawning, and a decrease during the autumn/winter gametogenesis and gonad maturation (Himmelman 1984, Scheibling and Hatcher 2007). Because feeding and gonad development exhibit seasonal cycles, gonad enhancement programs based on the use of formulated feeds should test feed efficiencies at different times of the year to establish temporal performance profiles that can be used to maximize commercial productivity.

In the present study, we carried out two gonad enhancement experiments with Newfoundland green sea urchins fed with a proprietary (Urchinomics) fish-meal-based feed. Both

experiments (1) were carried out in commercially available, low-flow containment systems adapted to hold urchins and quickly evacuate feces and other debris; (2) utilized urchins taken from barren grounds to ensure minimum initial gonad content; (3) differed in duration [4 or 8 wk] and timing of urchin collection from the field [just before or within the spawning period]; (4) exposed urchins to one of three relatively low water temperatures [1, 3, or 6°C]; and (5) measured various urchin bodily characteristics, feed consumption, feces production, gonad yield, and physiological condition (righting time). We also characterized gonad taste (assessed by a taste panel) following the longest experiment. This design allowed us to test the suitability of the feed for gonad enhancement under low operational requirements and at a time of the year when natural gonad growth is nearly arrested, potentially limiting feed effects.

2.2 MATERIALS AND METHODS

2.2.1 Collection and transfer of urchins to experimental tanks

The present study was carried out with green sea urchins (*Strongylocentrotus droebachiensis*) hand collected by divers on 10 February, 17 March, 13 April, 18 May, and 15 June, 2017, at depths of 6 to 10 m from an urchin barren in Bread and Cheese Cove (BCC, 47°18’30.8” N, 52°47’19.1” W), a semi-protected cove in Bay Bulls, southeastern Newfoundland (Canada). We chose this barren and these depths for collection because urchins there exhibit a relatively low (~3 to 7%) gonadosomatic index (GSI) year-round (P. Gagnon, personal observations), likely because of infrequent access to a limited detrital kelp subsidy brought in by storm waves in some years (Frey and Gagnon 2016). Sea urchins were transported to the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland in large containers filled with seawater. Upon arrival (~3 h after collection) the 600 urchins used in the gonad enhancement

experiment were transferred directly to the experimental tanks (see section 2.2.2) in the Dr. Joe Brown Aquatic Research Building, whereas the 50 individuals used to quantify baseline GSI (see section 2.2.5) were transferred to a separate holding tank. Experimental tanks and the holding tank were supplied with ~3 L min^{-1} of ambient flow-through seawater pumped from the adjacent Logy Bay. All sea urchins measured 45 to 55 mm in test diameter. We chose sea urchins of this size because they are sexually mature (Scheibling and Hatcher 2007) and dominated numerically at BCC at the times of collection.

2.2.2 Experimental approach

We used two experiments to study the effects of water temperature and temporal proximity to spawning on green sea urchin's feed consumption, feces production, gonad yield, righting time, and gonad taste. The two experiments had the same tank setup and used the same three temperature treatments (~1, 3, and 6°C, see below) but differed in timing of urchin collection and duration (Figure 2.1). Experiment 1 was carried out with green sea urchins collected in mid-February, i.e. right before their natural peak spawning period of March to May in Newfoundland (Himmelman 1978, Keats et al. 1984, Scheibling and Hatcher 2007), and lasted four weeks (excluding the initial week of acclimation). Experiment 2 was carried out with urchins collected in mid-April, i.e. during the spawning period, and lasted eight weeks (excluding the initial week of acclimation). Hence, for simplicity, urchins in Experiment 1 are hereafter termed "unripe urchins", whereas those in Experiment 2 are termed "ripe urchins". Even though no formal histological analysis was used to confirm the degree of ripeness, the initial amount of gonads in the urchins at the onset of both experiments suggest the latter designations are accurate (see results). Urchins in both experiments

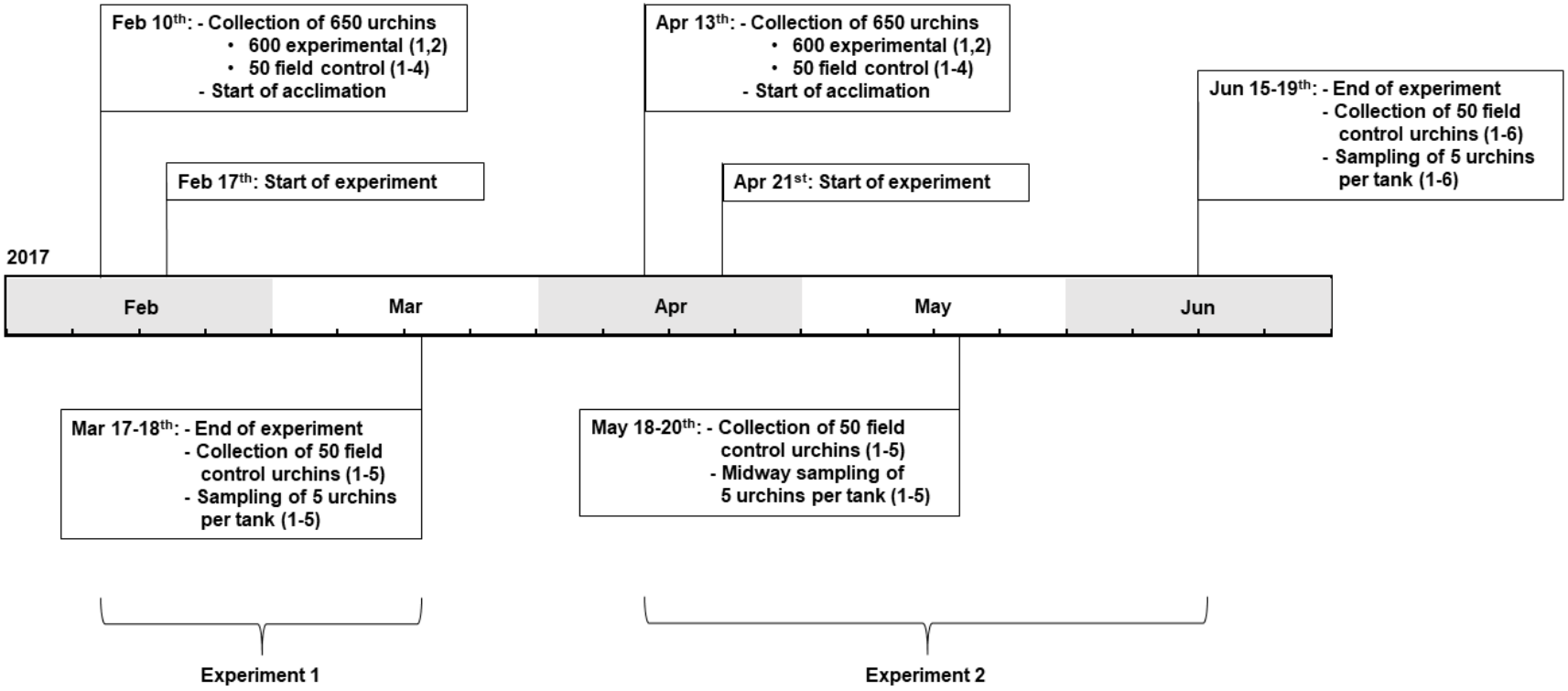

Figure 2.1 Timeline for the gonad enhancement Experiment 1 (4-wk long, carried out with unripe urchins) and gonad enhancement Experiment 2 (8-wk long, carried out with ripe urchins). Numbers in parentheses indicate characteristics measured: 1: Sea urchin body size (test diameter); 2: Sea urchin whole body wet weight; 3: Gonad wet weight; 4: Gonadosomatic index (GSI); 5: Urchin righting time; 6: Gonad taste.

were fed exclusively with the formulated feed Nofima V9 (Figure 2.2) according to protocols described below (see section 2.2.3).

The experimental setup for both experiments consisted of 30, 45-L cylindrical tanks organized in two adjacent rows of 14 tanks and one slightly off row of two tanks (Figure 2.3A-B). The room's shape, plumbing, and electrical power systems imposed this particular spatial arrangement of the tanks, with consequences on the spatial blocking of the temperature treatments among the tanks (Figure 2.3A-B). Each tank measured 58 cm (height) by 41 cm (diameter) and had a conical bottom overlaid by a perforated false bottom (Figure 2.3C-E) to collect and evacuate urchin feces quickly and easily. Final gamete development and gonad ripening/growth are not affected by photoperiod, so we chose a neutral 12/12 h daily light cycle throughout both experiments. During the lit portion of the cycle, ~35 lux of light (as measured with a portable lux meter, HI97500; Hanna Instruments) emitted by eight incandescent light bulbs (TCP Elite series LED 18 Watt / 5000K Incandescent dimmable; Technical Consumer Products Inc.) placed above the tanks (Figure 2.3A-B) penetrated water in each tank. Each tank was supplied with flow-through seawater (~3 L min^{-1}) throughout both experiments.

Both experiments were preceded by a 7-d acclimation phase during which urchins in the experimental tanks underwent gradual water heating and/or cooling cycles, up or down to the targeted experimental temperatures of ~1, 3, and 6°C. We chose to study these temperatures because they (1) are representative of sea surface temperature for the region during winter [1°C] and early spring [3°C]; and (2) provided the opportunity to examine the urchin's tolerance to a temperature that is more typical of early summer [6°C] (Blain and Gagnon 2013, Frey and Gagnon 2016). We kept water in all tanks at an ambient temperature of -0.5 to 1°C during the first five

Figure 2.2 C-shaped pellets (formulated feed Nofima V9) provided to urchins in the two urchin gonad enhancement experiments (photo: David Bélanger). Pellets are negatively buoyant and maintain their physical integrity for up to seven days in water below ~10°C. The curved shape of the pellets increases residence time within specifically designed urchin holding crates (SeaNest™, not shown).

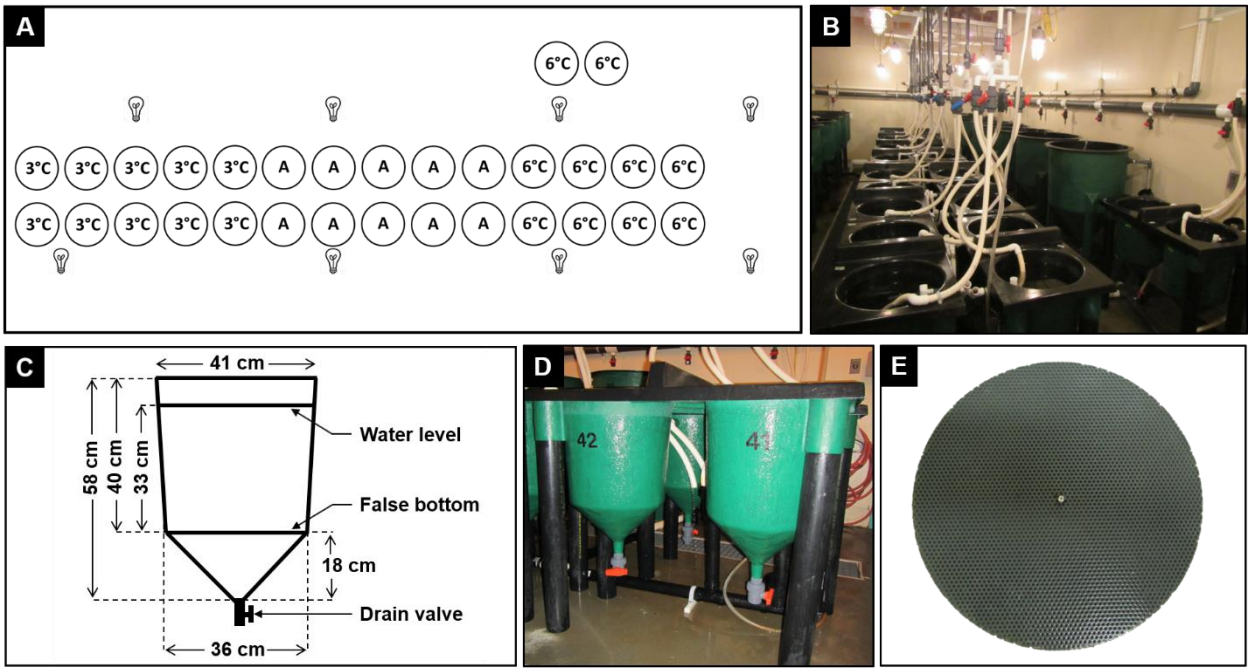

Figure 2.3 Experimental tank setup in the two urchin gonad enhancement experiments (schematics and photos: David Bélanger). (A-B) Schematic and image of the experimental room showing the spatial distribution of the tanks, assignment of tanks to water temperature treatments (where A is the ambient water temperature; ~1°C), and location of the eight incandescent light bulbs used to create the light environment. (C) Side view of a schematized experimental tank showing the physical dimensions and location of the false bottom and drain valve. (D) Close-up view of a few experimental tanks showing the [white] pipes supplying seawater and [red] drain valves used to purge feces and other debris accumulated at the bottom of the tanks. (E) Circular, perforated false bottom used to separate the top section of each tank from its conical bottom. The holes were small enough to let urchin feces pass through and accumulate in the conical bottom. Urchins were placed on the false bottoms at the onset of the experiments and had free, continuous access to the tank walls throughout both experiments.

days and heated it mechanically to ~3.5°C on the 6th day. On the 7th day, temperature returned to ~1°C in the tanks assigned to the ambient temperature treatment, whereas the 3° and 6°C tanks were switched on water heated to these temperatures. This gradual acclimation of urchins to their assigned temperature treatments helped reduce the likelihood of physiological stress and spawning during the following experimental trials.

Water temperature was recorded every 10 min throughout the acclimation phase and the two experiments with one temperature and light logger (HOBO Pendant; Onset Computer Corporation) placed on the bottom of one randomly chosen tank from each temperature treatment. A posteriori examination of water temperature data indicated that temperature in each treatment was quite stable throughout the experimental phases of both experiments, with a slight, inevitable increase over time in the ambient temperature treatment in Experiment 2 (Appendix A). Mean water temperature in the 1 (ambient), 3, and 6°C treatments was respectively 0.7, 3.2, and 6.4°C for Experiment 1, and 1.3, 3.1, and 6.3°C for Experiment 2 (Appendix A). For simplicity, hereafter we refer to each water temperature treatment by their corresponding nominal designations of 1, 3, and 6°C. Urchins were not fed during the acclimation phase to standardize hunger levels.

During each experiment's acclimation phase, the test diameter and whole body wet weight of each of the 20 urchins from each of the 30 experimental tanks were measured. Test diameter was measured with an electronic caliper (58-6800-4; Mastercraft), at the widest portion of the test, by compressing both tines gently but firmly in between the spines, to as close to the test as possible. Whole body wet weight was measured with a balance (PB3002-S/FACT; Mettler Toledo). Whole body wet weights were used to determine urchin biomass, which information was then used to calculate the initial amount of feed to be added to each tank (see section 2.2.3). Both experiments began with the addition of feed pellets to the bottom of each tank, followed by: (1) weekly

quantification of feed supply and consumption (see section 2.2.3); (2) biweekly quantification of urchin feces production (see section 2.2.4); (3) assessment of GSI midway through Experiment 2 and at the end of both experiments (see section 2.2.5); (4) measurement of urchin righting time midway through Experiment 2 and at the end of both experiments (see section 2.2.6); and (5) assessment of urchin gonad taste by a taste panel at the end of Experiment 2 (see section 2.2.7). Experiment 1 (4-wk long) ran from 10 February to 18 March, 2017, and Experiment 2 (8-wk long) ran from 13 April to 19 June, 2017 (Figure 2.1).

2.2.3 Feed supply and consumption

In both experiments, urchins were fed exclusively with the fishmeal-based formulated feed Nofima V9 (Figure 2.2), developed to accelerate gonad growth in sea urchins. The feed is manufactured as a C-shaped pellet after a proprietary production process (globally licensed to Urchinomics) that water-stabilizes pellets for up to seven days in water below ~10°C. Pellets are negatively buoyant, and their curved shape increases residence time within specifically designed urchin holding crates (SeaNest™, for images of this see James et al. 2017).

The feed was provided *ad libitum* to urchins, throughout both experiments. Both experiments began with the addition of ~38 g of feed pellets to the bottom of each experimental tank as per the distributor's recommended ratio of 37.5 g of feed per kg of urchins per week (each tank initially contained ~1 kg of urchins). At the end of each week, we collected the unconsumed feed (and feces, see section 2.2.4), which we dried at 50°C for 72 h and weighed. Minor adjustments to the frequency and amount of feed delivered to the tanks were necessary during the 2nd and subsequent weeks because of higher feed consumption in tanks at 3 and 6°C. This

approach ensured urchins in all tanks had continuous access to the feed, while keeping track of the exact amounts of feed consumed throughout both experiments.

Fresh, dry pellets inevitably contained some water. We used the following procedure to standardize and accurately relate fresh and unconsumed feed weights in Experiment 1. We weighed five samples of 25 pellets each, dried them at 50°C for 84 h, and weighed them again. The following equation was then used to determine, for each sample, the percent change in feed weight caused by water loss:

$$RWL = \frac{IW - FW}{IW}$$

where RWL is the relative weight loss (in %), IW is the initial (before drying) weight (in g), and FW is the final (after drying) weight (in g). Mean RWL (from the five samples) of 7.3% was then used as a correction factor to calculate the adjusted unconsumed feed dry weight with the following equation:

$$ADW = DW + (DW * 0.073)$$

where ADW is the adjusted unconsumed feed dry weight (in g), DW is the unconsumed feed dry weight (in g), and 0.073 is the correction factor. Net feed consumption (NFC) was then calculated as the difference between the initial weight of feed added and the adjusted unconsumed feed dry weight (ADW). To enable comparisons among tanks, we standardized weekly feed consumption per tank to weekly relative feed consumption per tank with the following equation:

$$RFC = \frac{NFC}{UB}$$

where RFC is the relative feed consumption (in g feed kg^{-1} urchin), NFC is the net feed consumption (in g), and UB is the urchin biomass (in kg).

We used the same procedure as above to standardize and relate fresh and unconsumed feed weights in Experiment 2, with an additional step prior to calculating RFC for weeks 5 to 8. This step accounted for the change in urchin biomass resulting from the removal of five urchins from each tank midway through the experiment (Figure 2.1). We used the following equation to adjust urchin biomass for each tank:

$$\mathrm{UB}_{adj} = \mathrm{UB}_i - total\ WBW$$

where UB_{adj} is the adjusted urchin biomass (in kg), UB_i is the initial urchin biomass (in kg), and total WBW is the total whole body weight (in kg) of the five urchins removed at the end of the 4th experimental week. Therefore, for Experiment 2, the denominator term for RFC calculation for weeks 1 to 4 and for weeks 5 to 8 was respectively UB_i and $\mathrm{UB}_{adj.}$

2.2.4 Feces production

In both experiments we quantified the amount of urchin feces twice in each experimental week. The first quantification was done ~3.5 days after adding new feed at the beginning of the week and the second quantification at the end of the week (when the unconsumed feed was collected). Feces (but not feed particles) were small enough to pass through the perforated false bottom and accumulate in the conical end of each tank. In each tank we forced all fecal material through the false bottom's holes by removing about half of the water from the tank, then gently pouring that water back in the tank with small buckets. We allowed feces to settle in the conical end for 15 min, then opened the drain valve to collect the accumulated mixture of feces and water. We sifted the mixture through a 500-µm sieve to retain all (and only) the feces, which we wet weighed in aluminum pans, dried at 50°C for 72 h, and dry weighed.

To enable comparisons among tanks, we standardized weekly wet and dry feces production per tank to weekly relative wet and dry feces production per tank with the following equation:

$$RFP = \frac{FP}{UB}$$

where RFP is the relative wet or dry feces production (in g feces kg^{-1} urchin), FP is the wet or dry feces production (in g), and UB is the urchin biomass (in kg).

To calculate RFP for weeks 5 to 8 of Experiment 2 we introduced the same additional step as that described for calculating feed consumption over the same weeks (see section 2.2.3). Accordingly, for Experiment 2, the denominator term for RFP calculation for weeks 1 to 4 and for weeks 5 to 8 was respectively UB_i (initial urchin biomass) and UB_{adj} (adjusted urchin biomass, i.e. after removal of five urchins per tank at the end of the 4^{th} experimental week).

2.2.5 Gonad yield

To standardize and compare gonad yields we used the gonadosomatic index (GSI), a widely used metric indicative of the gonadal proportion of a sea urchin's total biomass:

$$GSI\ (\%) = \frac{Gonad\ wet\ weight\ (g)}{Whole\ urchin\ wet\ weight\ (g)} * 100$$

We calculated GSI for each urchin in each of the following groups: (1) the 50 urchins collected from BCC prior to the start of each experiment to establish baseline GSI; (2) the 150 urchins [five per tank for each temperature treatment] sampled at the end of Experiment 1 and in the middle and at the end of Experiment 2; and (3) the 50 urchins collected from BCC at the end of Experiment 1 and in the middle and at the end of Experiment 2, used as procedural controls. We also measured with a caliper the test diameter of each of these urchins, while determining their

sex based on gonad visual characteristics (orangish and grainy in females, yellowish and milky in males).

2.2.6 Righting time

We used the time an urchin, manually flipped onto its aboral side, takes to flip back onto its oral side, commonly termed "righting time", to assess the overall physiological condition of urchins in both experiments, with an expected inverse relationship between righting time and physiological condition (Himmelman 1984, Kleitman 1941). The righting time of each urchin sampled for GSI assessment at the end of Experiment 1, and in the middle and at the end of Experiment 2, was measured prior to assessing GSI (150 experimental urchins [5 per tank] and 50 control urchins from BCC at each sampling time). We overturned each urchin onto its aboral side in the centre of a circular, seawater-filled plastic bucket (~15 cm high and 15 cm in diameter) and measured the time it took to right. Healthy urchins normally right within 1.5 to 2.0 minutes (Kleitman 1941). Therefore, we chose a conservative time of five minutes (300 seconds) as the maximum for an urchin to right. If the urchin did not right within 300 seconds, the individual was deemed unsuccessful at righting, thus possessing a poor physiological condition.

2.2.7 Gonad preference and taste

We assessed gonad taste three days after the end of Experiment 2 (Figure 2.1) in 72 experimental urchins from the three temperature treatments (24 urchins per treatment) and 24 urchins from the field, as per the protocol developed by Trueman (2019). Urchins to be used for the taste test were randomly selected from the 150 experimental and 50 field individuals sampled for sex, GSI, and righting time (Figure 2.1). We collected gonads from females only

because it was visually more appealing than that of males and is preferred on international markets (Pisces Consulting Limited 2014). Each female's gonad with the best colour and texture was soaked in clean seawater to remove any debris, then refrigerated in a labelled ice cube tray at 1°C to maintain freshness.

A taste panel was conducted at the Centre for Aquaculture and Seafood Development (C-ASD) of Memorial University of Newfoundland, who recruited a panel of 24 adult volunteers to participate on the panel. On the test day (19 June, 2017), we transported the gonad samples in coolers with ice from the OSC to the CAS-D facility (~15-min trip), where they were transferred immediately to refrigerators. The test ran over four consecutive sessions, each with six new panelists chosen randomly from the pool of 24. We asked each panelist to rank four unique gonad samples (one sample from each of the three groups of experimental urchins and one sample from field urchins), on two different scales. The first scale, which is equivalent to a classical hedonic test (Lawless and Heymann 2010), addressed the "overall preference" by incorporating all dimensions of gustatory perception, namely taste, smell, and texture. This scale ranged from 1 (dislike extremely) to 9 (like extremely) (Table 2.1). The second scale addressed exclusively the "taste" component, rating from 1 (very bitter) to 6 (perfectly sweet) (Table 2.1). Each panelist sat in individual booths surrounded by opaque dividers to prevent visual contact with other panelists. Panelists had up to 15 min to rank, from left to right, the four gonad samples ordered randomly side by side before them. Red lighting concealed true gonad colour and panelists did not know from which of the four groups of urchins each sample came. Panelists could taste each sample only once and rinse their mouth with water between samples to eliminate influence of repeated tastings or sample identity.

Table 2.1 Breakdown of the two scales (overall preference and taste) panelists used to rank gustatory appreciation of green sea urchin (*Strongylocentrotus droebachiensis*) gonads from experimental and field urchins at the end of Experiment 2 [adapted from Trueman (2019)].

Rank	Overall preference	Taste
1	Dislike extremely	Very bitter
2	Dislike very much	Bitter
3	Dislike moderately	Bland (not sweet, not bitter)
4	Dislike slightly	Sweet
5	Neither like, nor dislike	Very sweet
6	Like slightly	Perfectly sweet
7	Like moderately	
8	Like very much	
9	Like extremely	

2.2.8 Statistical analysis

2.2.8.1 Feed consumption

We used (nonparametric) Loess regression analysis to compare relative feed consumption (RFC in section 2.2.3) among urchins from the three experimental temperature treatments (1, 3 and 6°C) during Experiment 1 (4-wk) and Experiment 2 (8-wk). We chose this "local weighted regression" approach over a classical parametric test (e.g., ANOVA) because of its ability to fit a smooth curve (with confidence intervals [CIs]) through scattered points that would be otherwise difficult to model and interpret with a parametric curve (Zuur et al. 2009). With this approach, curves' segments with non-overlapping CIs are statistically different. However, segments with overlapping CIs may, in certain cases, also be statistically different. Therefore, a Welch's two-sample t-test was used to examine the statistical significance of differences in areas of overlapping CIs among temperature treatments, both within and between the two experiments. Each regression in Experiment 1 (N=120) and Experiment 2 (N=240) was based on the 10 weekly measures of RFC per temperature treatment (one measure per experimental tank).

2.2.8.2 Feces production

We used the same logic (Loess regression analysis) as above to compare relative feces production (RFP in section 2.2.4) among urchins from the three experimental temperature treatments (1, 3 and 6°C) during Experiment 1 (4-wk) and Experiment 2 (8-wk). Each regression in Experiment 1 (N=120) and Experiment 2 (N=240) was based on the 10 weekly measures of RFP per temperature treatment (one measure per experimental tank). Prior to running this analysis, we used simple linear regression analysis to examine the strength of the relationship between (1) RFC and RFP as calculated from feces wet weight [$p<0.001$; $R^2=0.35$ and $R^2=0.88$ for Experiment

1 and Experiment 2, respectively]; and (2) RFC and RFP as calculated from feces dry weight [p<0.001; R^2=0.43 and R^2=0.89 for Experiment 1 and Experiment 2, respectively]. Because the latter relationships presented the highest coefficients of determination, we applied the Loess regression analyses to RFP values calculated from feces dry weight.

2.2.8.3 Gonad yield

Because sea urchins from the field were not spatially blocked (by tank), 10 groups of urchins, each composed of 5 individuals chosen randomly from the pool of 50 field urchins, were created for each period considered. This procedure yielded an identical number of comparison groups and number of urchins within groups, therefore eliminating the need for special statistical treatment (Quinn and Keough 2002). A two-way ANOVA with the fixed factors Experiment (Experiment 1 and Experiment 2) and Treatment (the three water temperature treatments: 1, 3, 6°C, and urchins from the field) was used to compare gonadosomatic index (GSI) among experimental and field urchins at the end of Experiment 1 (N=40, including the 10 groups of field urchins) and end of the middle (4^{th}) week of Experiment 2 (N=40, including the 10 groups of field urchins). We ran a similar two-way ANOVA to compare GSI at the end of the middle (4^{th}; N=40, including the 10 groups of field urchins) and last (8^{th}; N=40, including the 10 groups of field urchins) weeks of Experiment 2, with the fixed factor Sampling time (4^{th} and 8^{th} weeks) instead of Experiment.

We also tested for differences in GSI (ΔGSI) among sea urchins between the start and end of Experiment 1, as well as between the start and middle, and start and end, of Experiment 2. The following equation was used to calculate ΔGSI for each urchin:

$$\Delta\text{GSI (\%)} = \left[\frac{\text{GSI}_E \text{ - } \text{GSI}_B}{\text{GSI}_B}\right] \times 100$$

where ΔGSI is the percent change in GSI for the period considered, GSI_E is the GSI at the end of the period, and GSI_B is the mean GSI of the 50 sea urchins collected from BCC before the start of Experiment 1 (4.0±0.3%) or Experiment 2 (7.9±0.7%). A two-way ANOVA with the fixed factors Experiment (Experiment 1 and Experiment 2) and Treatment (the three water temperature treatments: 1, 3, and 6°C, and urchins from the field) was used to compare ΔGSI at the end of Experiment 1 (N=40, including the 10 groups of field urchins) and end of the middle (4^{th}) week of Experiment 2 (N=40, including the 10 groups of field urchins). We ran a similar ANOVA to compare ΔGSI at the end of the middle (4^{th}; N=40, including the 10 groups of field urchins) and last (8^{th}; N=40, including the 10 groups of field urchins) weeks of Experiment 2, with the fixed factor Sampling time (4^{th} and 8^{th} weeks) instead of Experiment.

2.2.8.4 Righting time

We analysed urchin righting time with the same general approach used to analyse GSI and ΔGSI; we calculated the proportion of urchins (out of five individuals) that righted in each tank (yielding 30 proportions; one per tank) and for 10 groups of field urchins randomly chosen from the pool of 50 individuals. A two-way ANOVA was used with the fixed factors Experiment (Experiment 1 and Experiment 2) and Treatment (the three water temperature treatments: 1, 3, 6°C, and urchins from the field) to compare the proportion of urchins which righted within 300 s at the end of Experiment 1 (N=40) and end of the middle (4^{th}) week of Experiment 2 (N=40). We ran a similar ANOVA to compare the proportion of urchins which righted within 300 s at the end of the middle (4^{th}; N=40) and last (8^{th}; N=40) weeks of Experiment 2, with the fixed factor Sampling time (4^{th} and 8^{th} weeks) instead of Experiment.

Righting time was also analyzed as the average righting time per tank, with variable numbers of urchins (from 0 to 5) among tanks and groups of field urchins that righted in less than 300 s (a prerequisite for inclusion in the analysis, see section 2.2.6). We ran two two-way ANOVAs with the same structures as above (fixed factors Experiment or Sampling time, and Treatment) to compare the righting time of urchins which righted; one for between the end of Experiment 1 (N=38 [instead of 40] because we excluded two groups of field urchins in which none of the urchins righted) and end of the middle (4^{th}) week of Experiment 2 (N=40), and one for between the end of the middle (N=40) and last (N=40) weeks of Experiment 2.

2.2.8.5 Gonad preference and taste

We used multiple ordinal logistic regression ("MOLR") (McCullagh 1980) to examine the relationship between gonad overall preference rank (nine values from Dislike extremely [1] to Like extremely [9]) and the fixed factor Treatment (the three water temperature treatments; 1, 3, and 6°C, and urchins from the field). MOLR is designed for response variables on a scale where only relative ordering matters, and is beneficial for ranked data as it does not assume an equal distance between and among all numerical ranks. We used the same analysis for gonad taste rank (six values from Very bitter [1] to Perfectly sweet [6]). We applied both MOLRs to the raw data, with one ranking of overall preference and one ranking of taste for each of the four gonad samples (one sample per Treatment) tested by each of the 24 panelists (N=96 for each MOLR).

In all ANOVAs, simple linear regression analysis, and MOLRs we verified homogeneity of variance and normality of residuals by examining the distribution of the residuals and the normal probability plot of the residuals, respectively (Snedecor and Cochran 1994). These criteria were met in all analyses applied to the raw data. In all ANOVAs, the expected mean squares (EMS),

and corresponding F ratios and *p*-values, were calculated according to procedures for mixed models set by Quinn and Keough (2002). All MOLRs met the proportional odds assumption and no factors exhibited nominal or scalar effects (Brant 1990, McCullagh 1980). Differences among levels within a factor were determined with Tukey HSD multiple comparison tests for the ANOVAs and Bonferroni-adjusted pairwise comparisons for the MOLR (both based on least-square means; Quinn and Keough 2002, Sokal and Rohlf 2012). We used R 3.4.1 (R Core Team 2018) and a significance level of 0.05 for all analyses.

2.3 RESULTS

2.3.1 Thermal environment and urchin bodily characteristics

Water temperature in the tanks was, for each of the three temperature treatments, quite stable during the experimental phase of both Experiment 1 and Experiment 2, with a slight, yet inevitable increase over time in the ambient temperature treatment of Experiment 2 (Figure A.1). Temperature in the 1 (ambient), 3, and 6°C treatments was respectively 0.7, 3.2, and 6.4°C for Experiment 1, and 1.3, 3.1, and 6.3°C for Experiment 2 (Table A.1). In both experiments, test diameter of urchins in the tanks increased by only up to ~3% (at 6°C in Experiment 1; Table B.1), a result we expected given the relatively short duration of both experiments. Yet, body wet weight of urchins in the tanks varied more appreciably in both experiments; by up to ~10% (at 6°C) in Experiment 1, and up to ~7% (at 6°C) in Experiment 2 (Table B.1). These augmentations in body wet weight were largely caused by increases in gonad wet weight of up to 168% (at 6°C) in Experiment 1, and 164% (at 6°C) in Experiment 2 (Table B.1).

2.3.2 Feed consumption

In Experiment 1, relative feed consumption (RFC) was fairly stable during the first three weeks at 1°C, averaging 21.7±0.8 (SE) g feed kg^{-1} urchin, but then declined by ~30% (down to 15.1±0.8 g feed kg^{-1} urchin) in the last (4^{th}) week (Table B.2, Figure 2.4A). RFC at 3°C was a little more variable, yet significantly higher (by 23%) than at 1°C, except in the third week when it was similar between both temperatures, averaging 22.1±1.7 g feed kg^{-1} urchin (p=0.662). The most noticeable difference in RFC between both treatments was the decrease at 1°C versus increase at 3°C during the last week, resulting in a 90% higher feed consumption at 3°C (Figure 2.4A). RFC at 6°C was significantly higher than at 1 and 3°C in any given week (Figure 2.4A). RFC increased throughout the experiment, more so during the last two weeks, peaking at 51.0±0.6 g feed kg^{-1} urchin in the last week (Table B.2). Overall, urchins at 6°C consumed 1.5 and 2 times the feed consumed at 3 and 1°C, respectively (Table B.2).

In Experiment 2, the same general pattern of RFC variation applied at 1 and 3°C, with three distinct phases: (1) relatively low and stable RFC during the first three weeks; followed by (2) a 40 to 63% increase from the 3^{rd} to 5^{th} week; and (3) a 10 to 18% decrease from the 5^{th} to 8^{th} week (Table B.3, Figure 2.4A). RFC at 3°C was significantly higher, by 9 to 46%, than at 1°C in all weeks (Figure 2.4A). A similar pattern applied for the 6°C treatment, with the exceptions that Phase 2 (increase in RFC) began almost immediately into the 1^{st} week and Phase 3 (decrease in RFC) was much more abrupt, with an RFC decrease of 24% from the 5^{th} to 8^{th} week (Figure 2.4A). RFC in any given week was significantly higher at 6 than 3°C, by 38% (1^{st} week) to 94% (2^{nd} week) (Table B.3, Figure 2.4A). For all temperature treatments RFC was lowest in the 1^{st} (at 6°C) or 2^{nd} (at 1 and 3°C) week, with a minimum of 19.3±0.7 g feed kg^{-1} urchin at 1°C, and peaked in the 5^{th} week, with a maximum of 76.3±0.5 g feed kg^{-1} urchin at 6°C (Table B.3, Figure 2.4A).

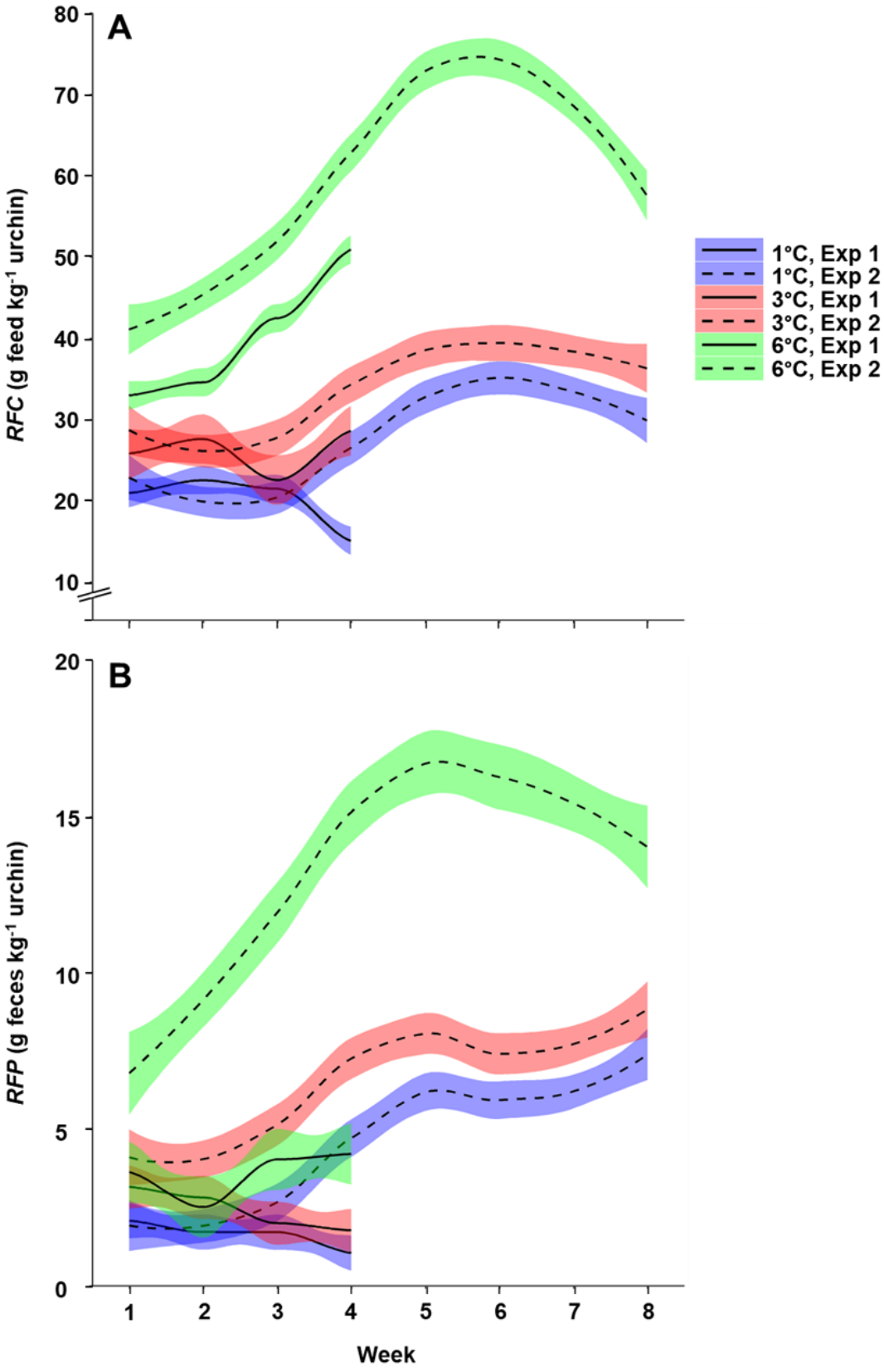
A
B
RFC (g feed kg-1 urchin)
RFP (g feces kg-1 urchin)
Week
1°C, Exp 1
1°C, Exp 2
3°C, Exp 1
3°C, Exp 2
6°C, Exp 1
6°C, Exp 2

Figure 2.4 Loess regression curves with ±95% confidence intervals (CI) showing the evolution of green sea urchin (A) relative feed consumption (RFC) and (B) relative feces production (RFP, based on feces dry weight) at each of the three water temperatures tested (1, 3, and 6°C) in Experiment 1 (4-wk long) and Experiment 2 (8-wk long). RFC and RFP were quantified once a week (n=10 for each temperature for each week).

Overall, urchins at 6°C consumed 1.8 and 2.2 times the feed consumed at 3 and 1°C, respectively (Table B.3). At 6°C, RFC in any of the first four weeks of Experiment 2 was significantly higher (by at least 20%) than in corresponding weeks of Experiment 1 (Figure 2.4A). At 3°C, RFC in Experiment 2 was also significantly higher (by at least 14%) than in corresponding weeks of Experiment 1 (Table B.2 and B.3), except in the 2nd week when it was similar between experiments (p=0.127). At 1°C, RFC was significantly higher (by at least 10%) in Experiment 2 than Experiment 1 in the 1st and 4th weeks, significantly higher (by 17%) in Experiment 1 in the 2nd week, and similar between both experiments in the 3rd week (p=0.359) (Tables B.2 and B.3, Figure 2.4A).

2.3.3 Feces production

Relative feces production (RFP) calculated from feces dry (not wet) weight best correlated with relative feed consumption (RFC) in both Experiment 1 (p<0.001, R^2=0.43) and Experiment 2 (p<0.001, R^2=0.89, Figure C.1), and hence was used in subsequent analyses. In Experiment 1, RFP at the two lowest temperatures declined steadily from the 1st to 4th (last) week; by 52% (from 2.1±0.2 [SE] to 1.0±0.2 g feces kg^{-1} urchin) at 1°C and by 44% (from 3.2±0.2 to 1.8±0.2 g feces kg^{-1} urchin) at 3°C (Table B.2, Figure 2.4B). RFP at 3°C was significantly higher (by at least 52%) than at 1°C, except in the 2nd and 3rd weeks when it was similar between both temperatures, averaging respectively 2.3±0.4 (p=0.081) and 1.9±0.4 (p=0.559) g feces kg^{-1} urchin (Table B.2). RFP at 6°C was significantly higher than at 1 and 3°C except for the 1st (p=0.259, 3°C) and 2nd (p=0.054, 1°C; p=0.615, 3°C) weeks. RFP at 6°C decreased by 31% in the first two weeks, but increased by 60% (to 4.0±0.5 g feces kg^{-1} urchin) in the 3rd week, with no further significant change

in the 4th week (Table B.2, Figure 2.4B). Overall, urchins at 6°C produced 1.5 and 2.3 times the feces produced at 3 and 1°C, respectively (Table B.2).

In Experiment 2, the same general pattern of RFP variation was observed at 1 and 3°C, with three distinct phases: (1) relatively low and stable RFP during the first three weeks; followed by (2) a 72 to 188% increase from the 3rd to 5th week; and (3) relatively high and stable RFP from the 5th to 8th week (Table B.3, Figure 2.4B). RFP at 3°C was significantly higher, by 10 to 139%, than at 1°C in all weeks, except in the 8th week (Figure 2.4B) when it was similar (p=0.089) between both temperatures. A similar pattern was observed for the 6°C treatment, except that Phase 2 (increase in RFP) began immediately after the 1st week and Phase 3 was characterized by a significant (19%) decrease in RFP from the 5th to 8th week, instead of the quasi plateau noted at 1 and 3°C over the same period (Figure 2.4B). RFP in any given week was significantly higher at 6 than 3°C, by 56% (8th week) to 150% (3rd week) (Table B.3, Figure 2.4B). For all temperature treatments RFP was lowest in the 1st (at 1 and 6°C) or 2nd (at 3°C) week, with a minimum of 1.8±0.1 g feces kg^{-1} urchin at 1°C, and peaked in the 5th (at 6°C) or 8th (at 1 and 3°C) week, with a maximum of 17.2±0.6 g feces kg^{-1} urchin at 6°C (Table B.3, Figure 2.4B). Overall, urchins at 6°C produced 2.0 and 2.9 times the feces produced at 3 and 1°C, respectively (Table B.3). At 6°C, RFP in any of the first four weeks of Experiment 2 was significantly higher (by at least 92%) than in corresponding weeks of Experiment 1. A similar pattern applied at 3°C, with significantly higher (by at least 34%) RFP in Experiment 2 in all but the 2nd week, when values were similar (p=0.185) between both experiments (Figure 2.4B). At 1°C, RFP in any given week was largely similar between Experiment 1 and the first half of Experiment 2, except for the 4th week when RFP was three orders of magnitude (330%) higher in Experiment 2 (Figure 2.4B).

2.3.4 Gonad yield

As required, urchin gonadosomatic index (GSI) was low at the onset of Experiment 1 and Experiment 2, averaging 4.0±0.3 (SE) and 7.9±0.7%, respectively. GSI at the end of the 4th week differed between the two experiments among temperature treatments (Table D.1). It was significantly higher (by at least 24% at 6°C) in Experiment 2 than Experiment 1 at any of the three controlled temperature treatments, while similarly low (<5%) in urchins from the field (Table B.1, Figure 2.5). In Experiment 1, final GSI peaked at 9.6% for 6°C and was at least 43% higher than the two other temperature treatments. It was similarly lowest, ~5%, in field urchins and urchins maintained at 1°C (Table B.1, Figure 2.5). In Experiment 2, GSI at the end of the 4th week (equivalent to Experiment 1's duration) was largely similar among the three controlled temperature treatments, ranging from 9.4% (at 1°C) to 11.9% (at 6°C), and at least 135% higher than in field urchins (Table B.1, Figure 2.5). GSI differed between the middle (4th week) and end (8th week) of Experiment 2 among temperature treatments (Table D.2). In the middle and at the end it was similarly lowest (~4.5%) in field urchins (Table B.1, Figure 2.5). At the end it peaked to 19.2% at 6°C and was at least 43% higher than the two other temperature treatments. For any given temperature treatment, GSI at the end was at least 35% higher (at 3°C) than in the middle (Table B.1, Figure 2.5).

As noted for GSI, change in GSI (ΔGSI) at the end of the 4th week differed between the two experiments among treatments (Table D.3), however with reversed trends (Figures 2.5 and 2.6) caused by the higher initial baseline GSI in Experiment 2 (7.9%) than Experiment 1 (4.0%). ΔGSI was significantly higher (by at least 176% at 3°C) in Experiment 1 than Experiment 2 at 3 and 6°C, and marginally higher (by 136%) at 1°C (Table B.1, Figure 2.6). Field urchins exhibited the lowest ΔGSI, with an increase of 11.7% in Experiment 1, and a decrease of 50.0% in

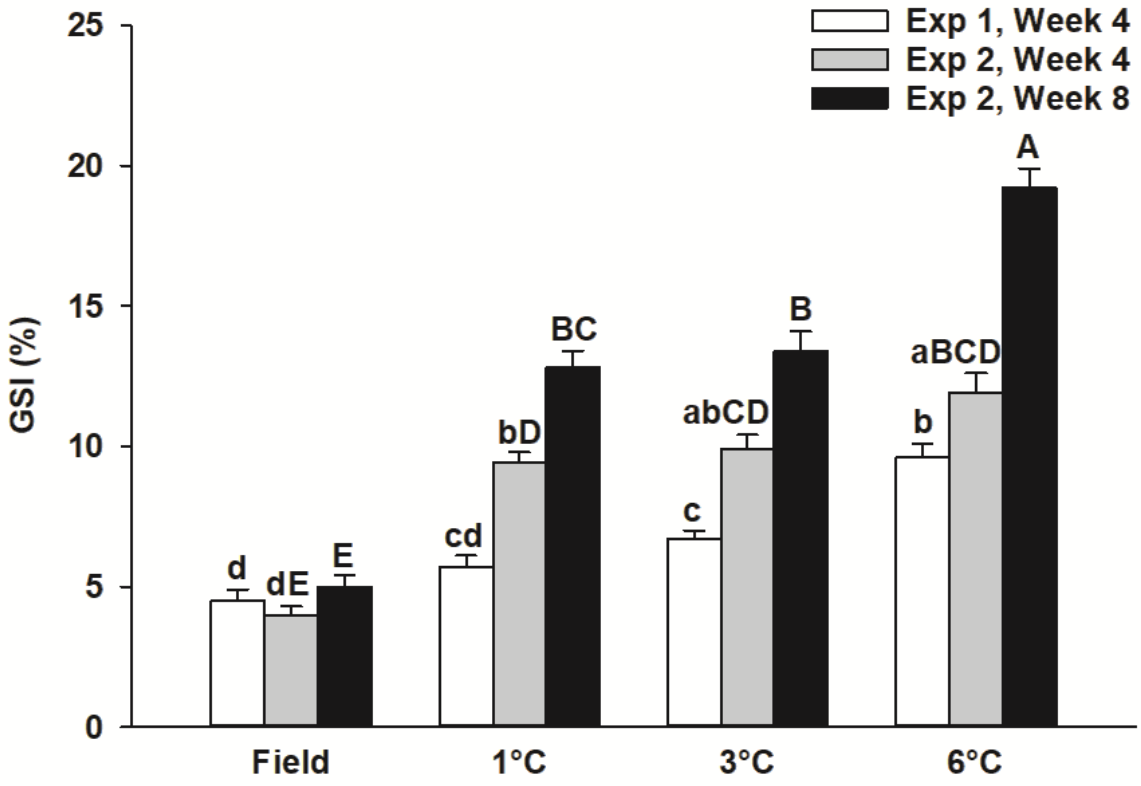

Figure 2.5 Mean (+SE) gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) taken from the field or exposed to one of the three temperatures (1, 3, and 6°C) at the end (Week 4) of Experiment 1 and in the middle (Week 4) and at the end (Week 8) of Experiment 2. Bars not sharing the same letter (lowercase for the analysis of Week 4 between Experiment 1 and Experiment 2; uppercase for the analysis of Week 4 and Week 8 in Experiment 2) differ statistically (LS means tests, $p<0.05$; n=10 for each bar).

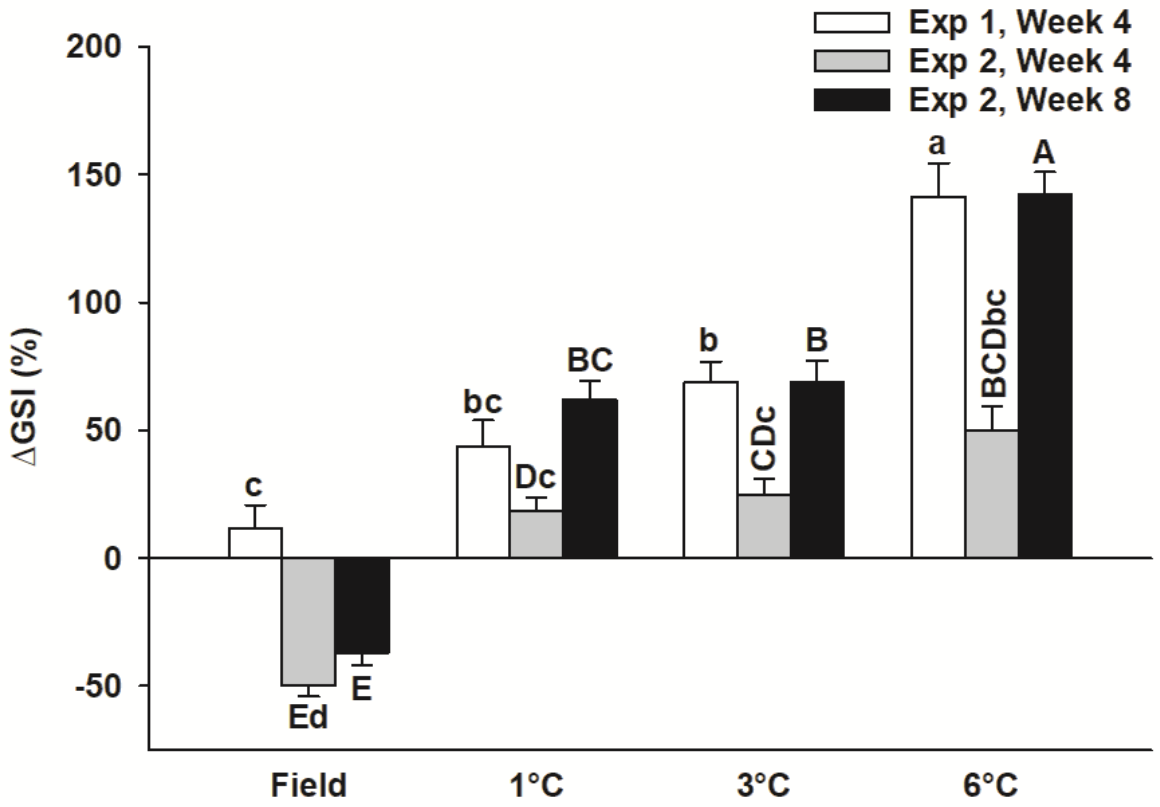

Figure 2.6 Mean (+SE) percent change (relative) in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) taken from the field or exposed to one of the three temperatures (1, 3, and 6°C) at the end (Week 4) of Experiment 1 and in the middle (Week 4) and at the end (Week 8) of Experiment 2. Bars not sharing the same letter (lowercase for the analysis of Week 4 between Experiment 1 and Experiment 2; uppercase for the analysis of Week 4 and Week 8 in Experiment 2) differ statistically (LS means tests, $p<0.05$; n=10 for each bar).

Experiment 2 (Table B.1, Figure 2.6). In Experiment 1, final ΔGSI peaked to 141.4% at 6°C and was at least 106% higher than the two other temperature treatments. It was lowest (43.9%) in urchins maintained at 1°C (Table B.1, Figure 2.6). In Experiment 2, ΔGSI at the end of the 4th week was largely similar among the three controlled temperature treatments, ranging from 18.6% (at 1°C) to 50.2% (at 6°C), and at least 137% higher than in field urchins (Table B.1, Figure 2.6). ΔGSI differed between the middle (4th week) and end (8th week) of Experiment 2 among treatments (Table D.4). In the middle and at the end it was similarly lowest (~ -45%) in field urchins (Table B.1, Figure 2.6). At the end it peaked to 142.3% at 6°C and was at least 107% higher than the two other temperature treatments. For any given temperature treatment, ΔGSI at the end was at least 176% higher (at 3°C) than in the middle (Table B.1, Figure 2.6).

2.3.5 Righting time

The proportion of urchins that successfully righted differed between the two experiments among treatments (Table D.5). At the end of the 4th week of Experiment 1, the proportion was similarly highest at 1, 3, and 6°C, ranging from 74 to 86%, and at least 2.6 times the observed 28% in field urchins (Figure 2.7). We noted a similar pattern at the end of the 4th week of Experiment 2, with the exception that the proportion of field urchins that righted was no different than that in urchins at the three temperature treatments, ranging from 80 (field urchins) to 100% (6°C) (Figure 2.7). Consequently, the proportion at the end of Experiment 1 was largely similar to that at the end of the 4th week of Experiment 2 for any given temperature treatment, yet was nearly three times that observed in field urchins from Experiment 2 compared to Experiment 1 (Figure 2.7). The proportion of urchins that successfully righted during Experiment 2 differed among treatments, but not between the 4th and 8th week (Table D.6). At the end of the 8th week, the proportion was

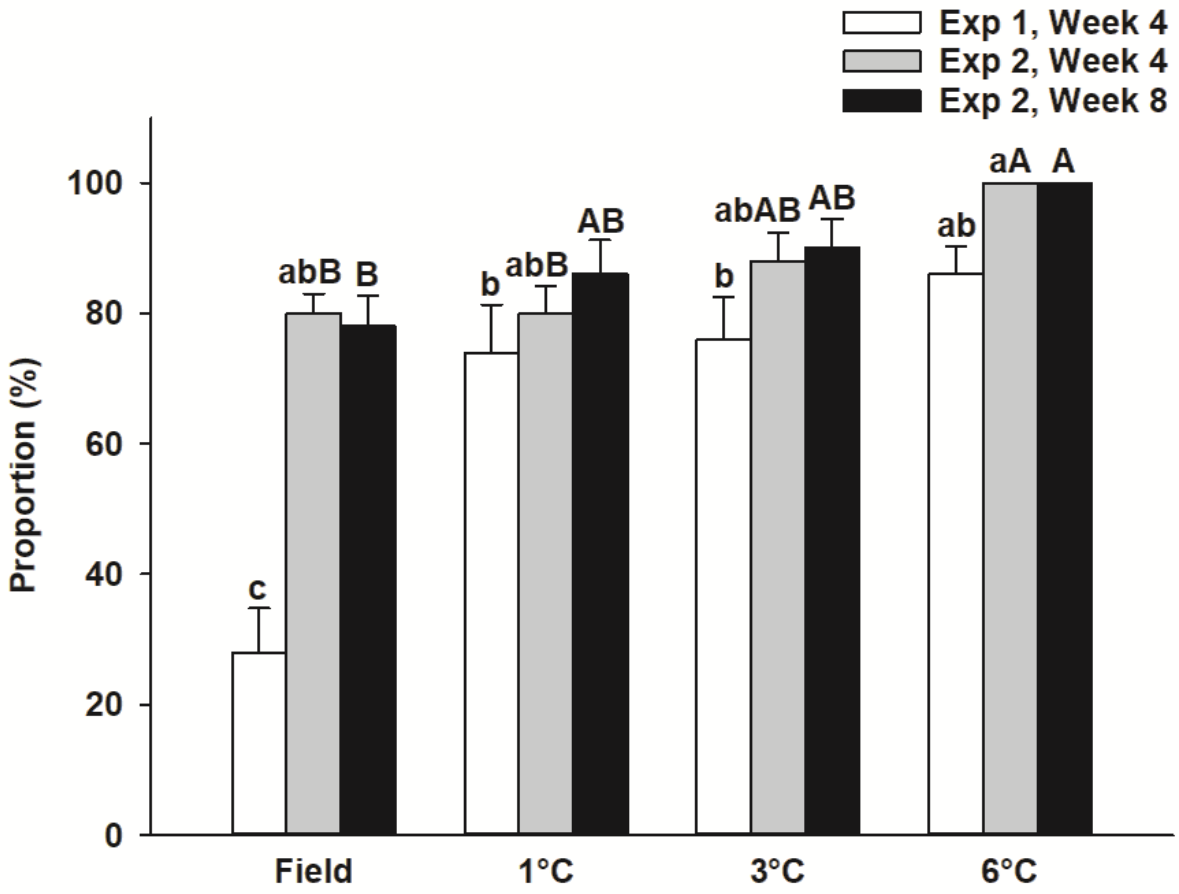

Figure 2.7 Mean (±SE) proportion of successfully righted green sea urchins (*Strongylocentrotus droebachiensis*) taken from the field or exposed to one of the three temperatures (1, 3, and 6°C) at the end (Week 4) of Experiment 1 and in the middle (Week 4) and at the end (Week 8) of Experiment 2. Bars not sharing the same letter (lowercase for the analysis of Week 4 between Experiment 1 and Experiment 2; uppercase for the analysis of Week 4 and Week 8 in Experiment 2) differ statistically (LS means tests, $p<0.05$; n=10 for each bar; all urchins righted at 6°C in Experiment 2, hence the absence of error bars).

similarly highest at 1, 3, and 6°C, ranging from 86 to 100%, and significantly higher at 6°C than the observed 78% in field urchins (Figure 2.7). Proportions were largely similar between the 4th and 8th weeks in field urchins and any of the temperature treatments (Figure 2.7).

Righting time at the end of the 4th week differed between the two experiments and among treatments (Table D.7). At the end of Experiment 1, righting time in the temperature treatments was similarly shortest at 3 and 6°C (133 and 154 s, respectively), whereas urchins at 1°C and from the field took significantly more time to right; up to 230 s (Table B.1, Figure 2.8). Righting times at the end of the 4th week of Experiment 2 were largely similar among the three controlled temperatures and field urchins, with the only significant difference of a ~30% increase between the shortest, 139 s at 6°C, and longest, 181 s at 1°C, times (Table B.1, Figure 2.8). With righting times of respectively 165 and 230 s, field urchins at the end of the 4th week of Experiment 2 righted significantly faster, by ~28%, than field urchins at then end of Experiment 1. However, righting times were similar between experiments in any of the three controlled temperature treatments (Table B.1, Figure 2.8). Righting time differed between the middle (4th) and end (8th) weeks of Experiment 2 among treatments (Table D.8). At the end, field urchins and those in the 1 and 3°C treatments exhibited similarly longest righting times of up to 159 s (field urchins); nearly twice that of the observed 86 s at 6°C (Table B.1, Figure 2.8). For any given temperature treatment, righting time at the end was at least 19% shorter (1°C) than in the middle. Field urchins took the same amount of time, ~162 s, to right in the middle and at the end (Table B.1, Figure 2.8).

2.3.6 Gonad preference and taste

The panelists' overall urchin gonad preference was largely similar, with no distinct patterns in the distribution of preference ranks among treatments (Table D.9; Figure 2.9). Yet, the majority

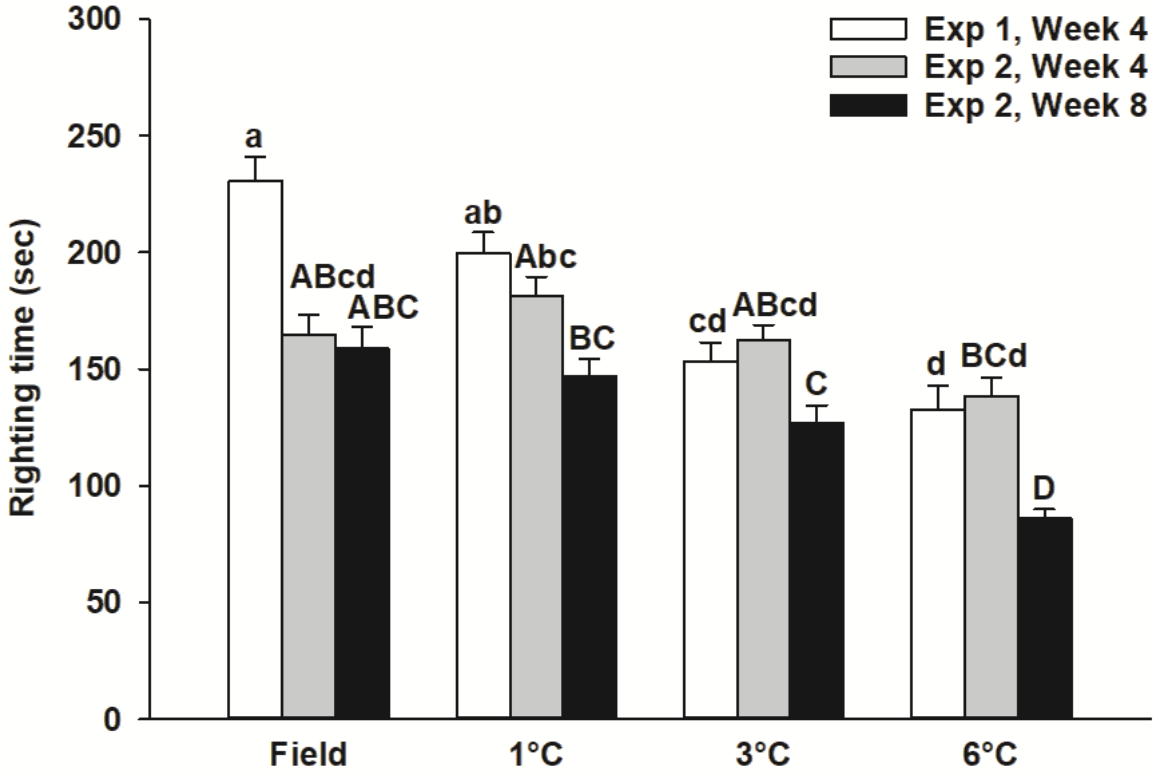

Figure 2.8 Mean (±SE) righting time of successfully righted green sea urchins (*Strongylocentrotus droebachiensis*) at the end (Week 4) of Experiment 1 and in the middle (Week 4) and at the end (Week 8) of Experiment 2. Bars not sharing the same letter (lowercase for the analysis of Week 4 between Experiment 1 and Experiment 2; uppercase for the analysis of Week 4 and Week 8 in Experiment 2) differ statistically (LS means tests, $p<0.05$; n=10 for each bar, except n=8 for field urchins in Week 4 of Experiment 1 because no urchins righted in two of the 10 groups).

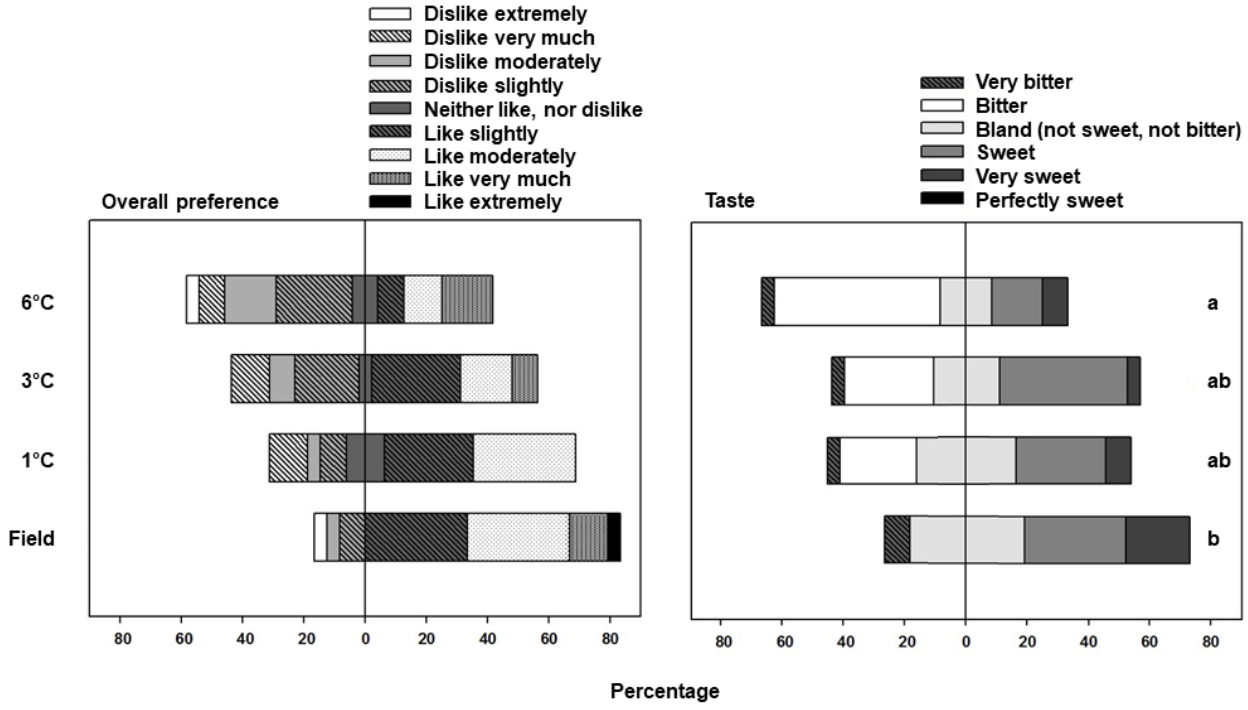

Figure 2.9 Percentage of gonads exhibiting overall preference ranks from Dislike extremely (1) to Like extremely (9) (left panel) and taste ranks from Very bitter (1) to Perfectly sweet (6) (right panel) in green sea urchins (*Strongylocentrotus droebachiensis*) taken from the field or exposed to one of the three temperatures (1, 3, and 6°C) in Experiment 2, as judged by panelists at the end of the experiment. Bars not sharing the same letter differ statistically (LS means tests, $p<0.05$; n=24 for each bar).

of panelists predominantly liked (slightly to extremely) gonads from field urchins (83%), followed by those from urchins that had been fed with the formulated feed in water at 1°C (63%) and 3°C (54%) (Figure 2.9). Interestingly, the majority disliked (slightly to extremely) gonads from urchins fed with the formulated feed at 6°C (54%) (Figure 2.9). The distribution of gonad taste ranks differed among treatments (Table D.9), with the majority of panelists rating gonads of field urchins as sweet to very sweet (54%) and gonads of urchins fed the formulated feed at 6°C as bland to very bitter (75%) (Figure 2.9). The distribution of gonad taste ranks of urchins fed the formulated feed at 1 and 3°C were similar to all other treatments, with the majority of panelists (~58%) rating them as bland to very bitter (Figure 2.9).

2.4 DISCUSSION

2.4.1 Feed consumption

Feeding in the green sea urchin (an ectotherm) generally increases with water temperature, largely because of the associated increase in metabolic activity (Brown et al. 2004, Carey et al. 2016, Cuthbert et al. 1995, Frey and Gagnon 2015, Garrido and Barber 2001, Scheibling and Hatcher 2007, Siikavuopio et al. 2006, Siikavuopio et al. 2008). Our finding that relative feed consumption (RFC) under our highest water temperature treatment (6°C) nearly doubled that under the two lower temperature treatments (1 and 3°C) in both Experiment 1 (which lasted 4 wk and utilized urchins collected from the wild just before the expected March-to-May natural spawning period) and Experiment 2 (which lasted 8 wk and utilized urchins collected during the spawning period), is consistent with this notion. The consistently low (at 1 and 3°C) or slightly increasing (at 6°C) RFC within the first two weeks of both experiments, along with a relatively sharp increase and subsequent drop (particularly at 6°C) in the remaining weeks of Experiment 2, indicate that

green sea urchin: (1) requires some acclimation to the formulated feed (Nofima V9), especially in water at or below 3°C; and (2) reaches a satiation point at ~6 wk beyond which the need to feed declines, more so at 6°C.

In eastern Canada, including Newfoundland, feeding in green sea urchin in natural habitats typically increases during and after the spring spawning gametogenesis (Himmelman 1984, Scheibling and Hatcher 2007). Siikavuopio et al. (2007a) report an increase in feed intake in Norwegian green sea urchins from late autumn/winter to late summer, followed by a decrease until autumn. Our finding that at 6°C, RFC in any of the first four weeks of Experiment 2 (which was carried out in spring) was at least 20% higher than in the corresponding weeks of Experiment 1 (which was carried out in late winter): (1) further corroborates the notion of a seasonal feeding cycle in green sea urchin; and (2) highlights the importance of integrating seasonal variability in feed consumption into commercial production strategies. The present study suggests that, other things being equal, gonad production costs could be lower in late winter than spring, given the smaller quantity of feed consumed during winter.

2.4.2 Feces production

In both experiments, temperature-specific patterns of relative feces production (RFP) were similar to those of RFC, except during the 2nd part of Experiment 2 at 1 and 3°C, when RFP continued to increase despite the declining RFC. Metabolism and digestion involve enzymatic processes that typically vary directly with temperature, as determined by the metabolic theory of ecology (MTE), meaning that urchin consumption and digestion should vary according to water temperature (Brown et al. 2004, Suskiewicz and Johnson 2017). Therefore, colder water in the present study presumably lowered urchin metabolic rates and delayed feed digestion and feces

evacuation, causing the discrepancy between RFC and RFP patterns, which potentially represents an operational limitation. Our observation that RFC and RFP (dry weight) were nevertheless highly correlated (more so in Experiment 2), is consistent with green sea urchin's patterns of feeding and feces production in natural habitats (Sauchyn et al. 2011, Sauchyn and Scheibling 2009). In echiniculture, fecal waste removal incurs some of the highest production costs (James and Siikavuopio 2015). The ability to predict feces production, therefore, is a major asset since the least time-consuming and most cost-effective feces removal methods can be planned in advance, and implemented based on effective urchin biomass. We showed that RFP calculated from feces dry weight better correlates with RFC than RFP calculated from feces wet weight. The up to 89% data fit between RFC and RFP (dry weight) for Experiment 2, speaks to the ability of accurately predicting the amount of feces produced based solely on the amount of feed provided to urchins.

2.4.3 Gonad yield

We showed that the Nofima V9 feed can enhance green sea urchin gonads to within the gonadosomatic index (GSI) market target of ~10 to 15% (Pisces Consulting Limited 2014) within only 4 wk in seawater at 6°C in both unripe (Experiment 1) and ripe (Experiment 2) urchins. As noted for RFC and RFP, GSI was always highest in urchins fed in seawater at 6 than at 1 or 3°C, though with less pronounced differences in GSI among temperatures in Experiment 2. We found that prolonging feeding for another 4 wk in ripe urchins enabled surpassing (with a GSI of ~19%) the market target at 6°C, while nearing the target's upper limit (with a GSI of ~13%) at the two lower temperatures. Taken together, these findings again speak to the key role of temperature in regulating urchin feeding and metabolism (Carey et al. 2016, Cuthbert et al. 1995, Frey and Gagnon 2015, Garrido and Barber 2001, Scheibling and Hatcher 2007, Siikavuopio et al. 2006,

Siikavuopio et al. 2008), and the importance of choosing water temperature according to operational capabilities, for gonad enhancement purposes. As we showed, more feed is consumed and more feces are produced in seawater (at least within the 1-to-6°C range), yet with the much-desired outcome of a higher gonad yield achieved in less time than in colder water. In a companion study, Trueman (2019) examined three locally abundant kelp species as feed options for gonad enhancement in Newfoundland green sea urchins and showed that at least 12 wk of feeding during summer are required to enhance gonads to within the GSI market target. Our results therefore show that the Nofima V9 feed enables satisfactory gonad enhancement in ~50% less time than with entirely natural (kelp) diets, when undertaken directly before and during spawning.

Our analysis of the differences in GSI (ΔGSI) between the start and end or middle of each experiment highlighted the merits of utilizing the feed with urchins from the wild that have the lowest possible GSI to begin with. Indeed, at 6°C, urchins in Experiment 1, which had an initial GSI of ~4%, exhibited a ΔGSI of ~140% after 4 wk, whereas those in Experiment 2, which had an initial GSI of ~8%, exhibited a ΔGSI of ~50% after 4 wk, and of ~142% after 8 wk. This nearly twice higher rate of GSI increase in unripe compared to ripe urchins may be explained by the deceleration of gonad growth that occurs during the reproductive cycle throughout the late winter/spring period, over which both experiments were carried out (Scheibling and Hatcher 2007). It could also be that with a final GSI of ~19% (see above) the internal space available to store additional gonad tissue became increasing limiting during Experiment 2 (P. Gagnon, personal observations), contributing to the observed lower ΔGSI. Interestingly, we noted negative ΔGSI (of ~ -45%), which is indicative of spawning, in wild urchins we collected for use as procedural controls in the middle and at the end of Experiment 2, whereas spawning possibly occurred in only one urchin at 1°C in the middle of Experiment 2.

2.4.4 Righting time

Our finding that the majority (over 74%) of urchins righted (i.e. flipped from their aboral to oral side) within 300 s at any of the three water temperatures in both experiments (more so in Experiment 2), indicates that the Nofima V9 feed sustained good physiological condition. The much lower proportion (28%) of wild urchins collected at the end of Experiment 1 that righted, suggests that urchins in the wild were physically less active at this time of the year, possibly as a result of the chronic low sea temperature (~2°C or less; Caines and Gagnon 2012, Frey and Gagnon 2016, Han et al. 1999) or food (e.g., kelp and other seaweed subsidy) shortage in the barrens from which they were taken (Blain and Gagnon 2014, Frey and Gagnon 2015). Kleitman (1941) concluded that temperature and righting time are generally inversely related in echinoderms, whereas Wei et al. (2016) show that feeding and righting time are inversely related in the sea urchin *Glyptocidaris crenularis*. Our results showed similar trends, with (1) an overall decrease in righting time with simultaneous increases in water temperature and RFC at the end of Experiment 1 and 4th week of Experiment 2; and (2) significantly shorter righting times at the end than in the middle of Experiment 2 at all temperatures. Urchin mortality was very low (<1%) in Experiment 1, and null in Experiment 2, which further attests to the suitability of the feed and rearing conditions for green sea urchin gonad enhancement.

2.4.5 Gonad preference and taste

A common issue with formulated feeds is the generally suboptimal gonad taste, colour, and texture they impart (Pearce et al. 2002, 2003, 2004, Prato et al. 2018, Siikavuopio et al. 2007b). In the present study, taste panelists preferred gonads of urchins taken from the wild over those of urchins fed with the Nofima V9 feed. As with RFC and RFP, gonad bitterness in feed-fed urchins

increased with water temperature, with twice as much feed consumed, and twice as many unfavorable gonad bitterness judgements, at 6 than at 1°C. Our results therefore demonstrate a direct relationship between feed consumption and gonad taste, whereby consumers of urchin gonads are increasingly likely to turn down the product as urchins consume larger quantities of the feed. The Nofima V9 feed is fishmeal-based and proprietary (Urchinomics), preventing disclosure of its detailed formulation. The Urchinomics Sensory Committee (UCS; an international committee of experts in the sea urchin industry) independently tasted gonads of green sea urchins fed with the Nofima V9 feed (B. Tsuyoshi Takeda, personal communications). The committee concluded that gonads presented the targeted sweet and creamy flavor; however, left an unpleasant bitter aftertaste. Gonad colour and texture however met market's expectations, which, together with our results, indicate that only modifications involving taste are required to improve the feed's formulation so that it imparts the much-desired umami aftertaste (Stefánsson et al. 2017, Yamaguchi 1991). Such an improvement is advisable, considering that the price of low-quality gonads on global sea urchin markets only reach ~1/3 of that of high-quality gonads (Explorations Unlimited Inc. 2006).

2.4.6 Conclusion and future research directions

We showed that the Nofima V9 feed represents a viable feed option to enhance green sea urchin gonads to within the GSI market target of ~10 to 15% (Pisces Consulting Limited 2014) in only ~4 to 6 wk (the present study) or ~50% less time than with a diet of purely kelp (Trueman 2019). As also demonstrated, water temperature exerts a considerable influence on feed consumption, feces production, and gonad yield, which all nearly double at 6 compared to 1°C. Temporal proximity to spawning does not appreciably impact the urchin's overall physiological

condition, nor does it represent an obstacle to urchin gonad production, with virtually the same outcomes in unripe and ripe urchins. The only shortcoming is the bitter gonad taste imparted by the feed, which increases with increasing seawater temperature and feed consumption. Follow-up studies with urchins initially fed the feed and switched to a diet of kelp should be carried out as a potential strategy to enhance gonad taste. A companion study examining drivers of gonad taste (amino acid profiles) in Newfoundland green sea urchins fed with the Nofima V9 feed or either of two kelp-based variants of the feed, highlights the benefits of switching from an animal-protein-based, to a kelp-protein-based, formulation (Pellerin 2020). Further studies in this area are advisable to further perfect the feed formulation and gonad taste. Nevertheless, a major advantage of the Nofima V9 feed, low-flow containment system (conical tanks with false bottoms and non-intrusive feces/debris evacuation system), and stable thermal and light environments used in the present study, is the ability to maintain high gonad production, while avoiding spawning, even at a time of the year when natural gonad growth is nearly arrested and urchins in the wild are largely spawning. Similar experiments at different times of the urchin's reproductive cycle, and covering a broader range (essentially higher) of seawater temperatures, should also be carried out to establish the feed's temporal and thermal performance profiles, which can then be used to optimize commercial production.

CHAPTER III

Effects of stocking density on feed consumption, aggregation, and gonad yield in green sea urchin (*Strongylocentrotus droebachiensis*) in a tiered raceway system

ABSTRACT

In recent years, sea urchin gonads (roe/uni) have increased in popularity worldwide. The high value of urchin gonads on global seafood markets along with increasing demands have led to the development of formulated-feed-based gonad enhancement programs. The abundance of the green sea urchin, *Strongylocentrotus droebachiensis*, along the coast of eastern Canada represents an untapped resource with great potential for gonad enhancement. We carried out a 7-wk gonad enhancement experiment with Newfoundland green sea urchins fed with the latest improved version of a proprietary formulated feed. The experiment was carried out in a high-flow, tiered raceway system at a water temperature of 6°C, with urchins taken from barren grounds to ensure minimum initial gonad content. Urchins were fed at three different stocking densities (2.5, 6.5, and 10.5 kg urchins m^{-2}), and urchin feed consumption, aggregation, and gonad yield were recorded at set times throughout the experiment. We also carried out concurrent trials in the raceway with urchins fed a natural kelp diet (*Laminaria digitata*) for gonad yield comparisons. We show that the feed represents a viable option to enhance gonads from a GSI of ~4% to above the GSI market target (~10 to 15%) in less than 7 wk in the raceway, and to achieve a higher GSI than the natural kelp diet. Feed consumption varied according to position within the raceway, with the lowest levels in the most downstream positions. Aggregation patterns were also influenced by raceway position, as well as urchin density, and reflected behaviours observed in the natural environment. Despite variations in feed consumption and aggregation patterns, GSI remained consistently high across all urchin densities and raceway positions, and mortality was found to be negligible, indicating that urchins can be stocked at the highest urchin density throughout the raceway to maximize production. Findings from this study demonstrate the efficiency of the feed in combination with the tiered raceway system, achieving marketable green sea urchin gonads in just a few weeks time.

3.1 INTRODUCTION

Green sea urchin (*Strongylocentrotus droebachiensis*) has become a popular organism for aquaculture in recent years as there is an increasing demand for green sea urchin gonads (uni/roe) on the seafood market (Stefánsson et al. 2017). Although wild urchins are harvested for their gonads in coastal areas throughout the world (Andrew et al. 2002, Keesing and Hall 1998), gonad enhancement in land-based facilities is increasingly favored to control environmental conditions and maximize production, while optimizing quality (Daggett et al. 2006). Urchins with optimal gonad quality and yield have the most value on international seafood markets (Explorations Unlimited Inc. 2006). Industry research is therefore focussed on the development of methods that will optimize land-based urchin gonad production.

Macroalgae, in particular kelp, are the main natural food source of sea urchins (Scheibling et al. 1999), however harvesting macroalgae throughout the year can be costly and logistically challenging due to seasonal variation in kelp quality and quantity (Carrier et al. 2017, Frey and Gagnon 2015, Himmelman 1984). Another option is to use formulated urchin feeds, developed to enhance urchin gonad production for the seafood industry; a diet of formulated feed is beneficial since consistent kelp harvesting is avoided, and marketable urchin gonad size is achieved more rapidly compared to a diet of kelp (Azad et al. 2011, Carrier et al. 2017, Pearce et al. 2002), which can take between 12 to 34 weeks (Trueman 2019).

In addition to diets, various sea urchin containment systems can be used to maximize production. Ideal urchin containment systems optimize space use, water use, feed access, and gonad development, while limiting mortality (Daggett et al. 2006). Aquaculture containment systems used for sea urchin gonad production typically involve either tanks or raceways, however research is still needed on optimal containment system design (Daggett et al. 2006, James and

Siikavuopio 2015). A raceway typically is a trough several metres long and a few 10s of centimeters wide and deep, with water entering at one end or along the length of the trough, and draining out the other end (Christiansen and Siikavuopio 2007, Daggett et al. 2006, James and Siikavuopio 2012, Siikavuopio et al. 2006). Tiered raceways are composed of several superimposed (stacked) troughs, with water entering the system in the top tier and flowing down (generally by simple gravity) successively into the lower tiers (Daggett et al. 2006, Grosjean et al. 1998, Le Gall 1990).

Tiered raceways present several advantages over other tank designs, including better vertical space utilization and water use because the same water flows successively through multiple tiers and can all be evacuated at the same location (Daggett et al. 2006). However, water quality may vary from one tier to the next. For example, dissolved oxygen (O_2) may be reduced in the lower tiers as a result of oxygen use by urchins located in the upper, preceding tiers. Water temperature in the lower tiers may also be higher as water warms up down the way, and pH may be lower due to carbon dioxide (CO_2) accumulation in the water from urchin respiration and the release of carbonic acid (H_2CO_3) during test growth (Grosjean et al. 1998, Siikavuopio et al. 2007e). Feces, unconsumed feed particles, and other debris may also be transported by water from one tier to the next, accumulating in the lower tiers and potentially contaminating the water and urchins (Mortensen et al. 2012). These potential sources of water quality degradation therefore must be considered for urchin gonad enhancement purposes.

Urchins in smaller individual tanks generally congregate on vertical surfaces (sides) or in corners, reducing access to feed (Daggett et al. 2006, Devin 2002). Because shallow raceways present significantly less vertical surface space, urchins are forced onto the horizontal (bottom) surfaces, therefore enhancing feed access (Daggett et al. 2006, Devin 2002). The formation of

dense aggregations in tanks mirrors the urchin's natural aggregation behavior at the lower edge of kelp beds in the wild (Gagnon et al. 2004, Himmelman 1984, Scheibling et al. 1999, Steneck et al. 2002). Green sea urchin also reduces displacement and attachment to vertical surfaces, while forming larger and tighter aggregations as wave velocity increases and urchin population density increase (Frey and Gagnon 2016). The development of productive urchin gonad enhancement programs in raceway systems therefore requires the integration of accurate knowledge and understanding of (1) the urchin's natural feeding and aggregation behavior and response to water flow; and (2) the physical characteristics and influence of the raceway on water quality and flow.

In the present study we carried out a 7-wk experiment with Newfoundland green sea urchins (*S. droebachiensis*) during which we fed urchins a formulated feed at three different stocking densities (2.5, 6.5, and 10.5 kg urchins m^{-2}) in a tiered raceway, and recorded feed consumption, aggregation, and gonad yield at set times throughout the experiment. This formulated feed is an improved version of the feed tested in Chapter II. We also carried out concurrent trials with urchins fed kelp (*Laminaria digitata*) in the raceway for gonad yield comparisons. The main goals were to: 1) determine the differences in feed consumption among stocking densities and locations within the raceway over time, 2) determine aggregation patterns over time and differences in aggregations among stocking densities and locations within the raceway, 3) determine the optimal stocking density and location within the raceway that maximizes gonad yield, and 4) compare gonad yield among feed-fed and kelp-fed urchins in the raceway.

3.2 MATERIALS AND METHODS

3.2.1 Collection and transfer of urchins

Green sea urchins (*Strongylocentrotus droebachiensis)* were hand collected by divers from a semi-protected cove in Bay Bulls, Newfoundland called Bread and Cheese Cove (BCC, 47°18'27.5" N, 52°47'16.7" W) on 15 and 19 of January, and 15 March, 2018. Urchins were collected from the barren in BCC from a depth of 6-10 m since these urchins exhibit a relatively low (~3 to 7%; P. Gagnon, personal observations) gonadosomatic index (GSI) year-round (a prerequisite for optimal feed performance as explained in section 2.2.1, Chapter II). Fifty (50) additional urchins were collected from the kelp bed in BCC from a depth of 1-3 m for use as controls in urchin GSI comparisons. Sea urchins were transported in large containers (~100 L) filled with seawater to the Dr. Joe Brown Aquatic Research Building at the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland. Approximately 2,200 urchins from the barren were placed directly in the raceway (~4 h after collection) for approximately 10 days of acclimation before the start of the trials. The remaining urchins from the barren (collected to keep as replacements for mortalities in the raceway), as well as the 50 urchins from the kelp bed were kept in separate flow-through holding tanks at the OSC with ambient seawater pumped from the adjacent Logy Bay. Prior to the start of trials, urchins outside the range of 40-60 mm were removed from the raceway, and the remaining urchins were distributed in the raceway compartments by stocking density (see section 3.2.2). Urchins 40 to 60 mm in test diameter were selected for the experiment because they are sexually mature (Scheibling and Hatcher 2007) and dominated numerically at BCC.

3.2.2 Raceway system and experimental design

The raceway was a three-tiered structure approximately 5.8×1.5×1.7 m (L×W×H; Figure 3.1). Each tier was made of a fiberglass trough with the straight, parallel upstream and downstream segments each measuring 4.3×0.6×0.1 m (L×W×H), connected by a semi-circular turn 1.5×0.6×0.1 m (L×W×H) (Figure 3.2). A semi-circular turn at the start of the upstream straight segment collected water for the tier (Figure 3.2). This raceway set-up involved a flow-through system with water entering the top tier from a header tank, then falling from the end of one tier into the beginning of the next until flowing out the end of the bottom tier (Figures 3.1 and 3.2). Each straight segment of the raceway was at a slight incline (~1% slope), which triggered water flow by gravity. Water temperature was maintained at ~6°C (see section 3.2.3), since this temperature was found to maximize gonad growth in Chapter II, and water flow was maintained between 70 and 80 L min^{-1}.

The top two tiers of the raceway contained urchins fed exclusively a formulated feed (see section 3.2.4). Each straight segment of the top two tiers (Figure 3.2) was divided into six compartments (12 compartments per tier; 24 in total) of 0.7×0.6 m (L×W; ~0.38 m^2), each delimited by 60.0×1.3×7.0 cm (L×W×H) separators over and under which water could flow to the next compartment. These compartments were used to contain and separate urchins at the three different stocking densities tested (see below). Compartments were spatially organized into groups of three adjacent compartments (Figure 3.2), each assigned to one of the three urchin stocking densities. The placement of each density within each group of three compartments was randomized for each trial. The turns connecting the straight segments in each tier were divided into three compartments of ~0.32 m^2, divided by two 1.3 cm wide separators (Figure 3.2). The six

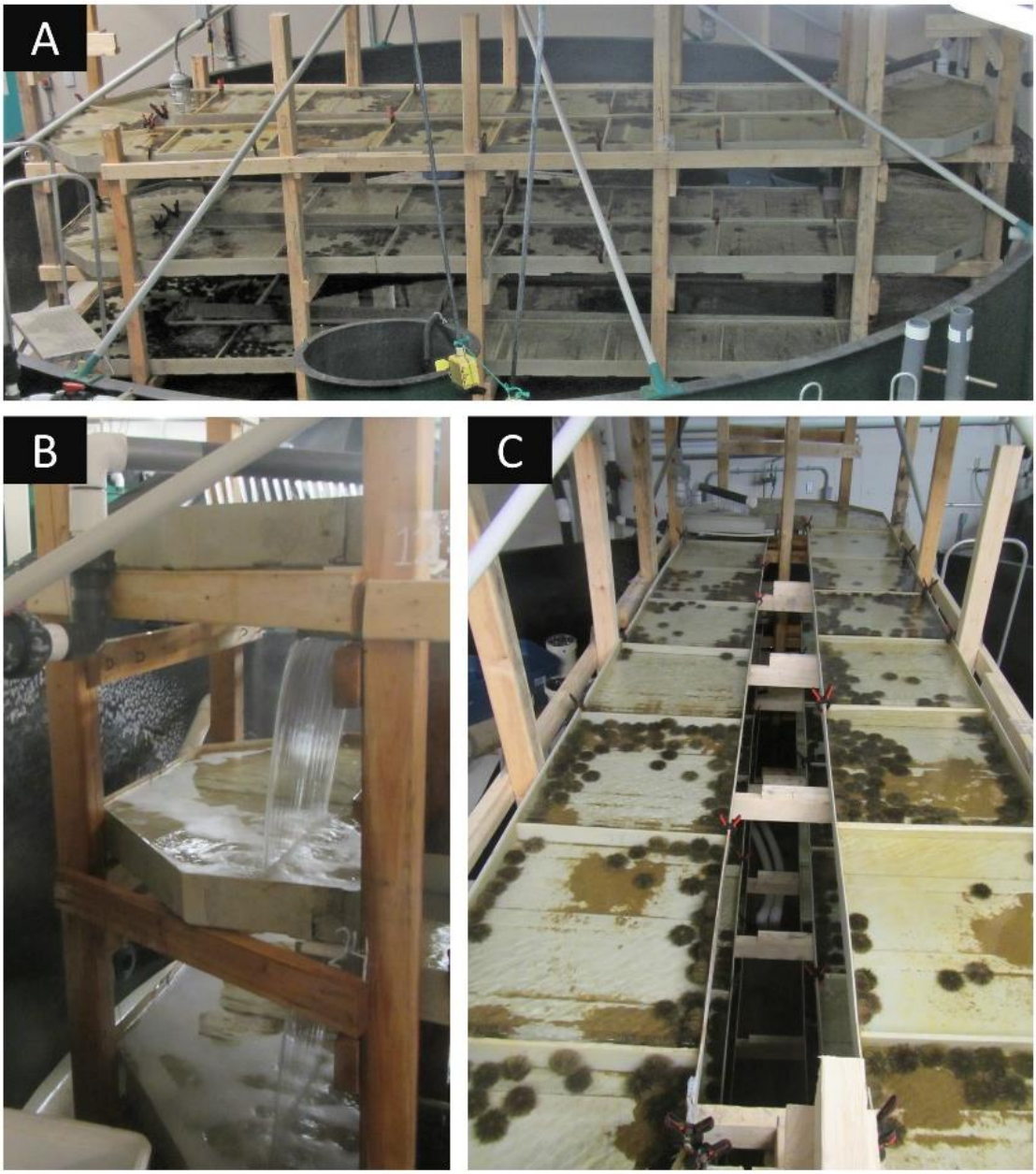

Figure 3.1 Images of the three-tiered raceway showing (A) the entire raceway with the top, middle, and bottom tiers; (B) water entering the top tier from a header tank and then falling from the end of the top tier into the middle tier, and from the end of the middle tier into the bottom tier; and (C) the layout of the compartments in the top tier containing green sea urchins (dark green; *Strongylocentrotus droebachiensis*) and patches of feed and/or feces (beige).

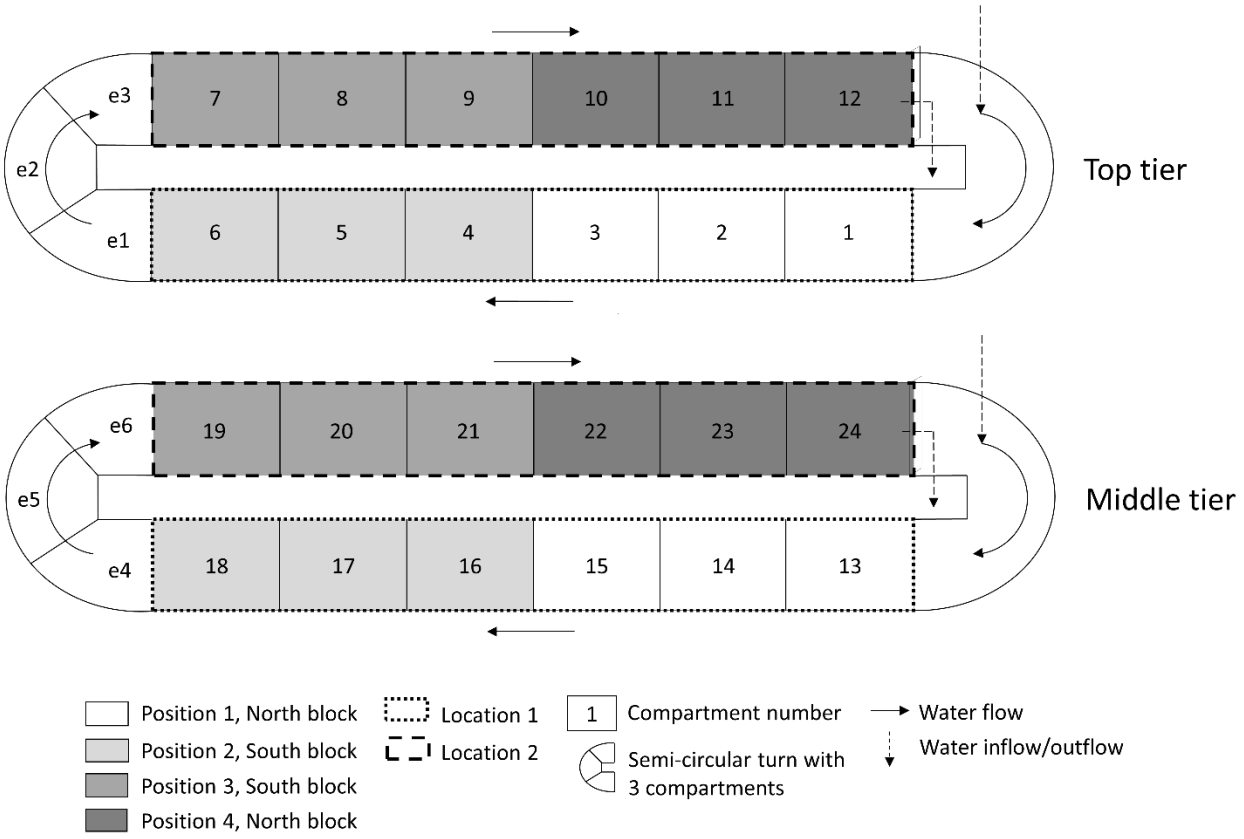

Figure 3.2 Schematic of the top tier (compartments 1-12; semi-circular turn compartments e1-e3) and the middle tier (compartments 13-24; semi-circular turn compartments e4-e6) of the raceway in which urchins were fed the formulated feed. Each tier had two Locations (1; upstream straight segment, and 2; downstream straight segment), four Positions (1 [furthest upstream in Location 1] to 4 [furthest downstream in Location 2]), and two Blocks (north [Positions 1 and 4] and south [Positions 2 and 3]). Each group of three adjacent straight compartments (1-3, 4-6, etc.) contained one of each of the three urchin stocking densities, and each turn compartment contained the intermediate density. Water originally flowed into the top tier from a header tank, then fell from the end of the top tier into the middle tier, and from the end of the middle tier into the bottom tier (see Figure 3.3 for bottom tier schematic).

compartments in the turns housed the intermediate urchin density (same as that in the straight segments), to test a standard density between the low and high extremes.

Three urchin stocking densities (low, intermediate, and high) were tested to compare the effects of raceway stocking density on urchin feed consumption, aggregation, and gonad yield. A low density of 50 urchins m^{-2} (2.5 kg urchins m^{-2}; 19 urchins per compartment) was selected based on the urchin density found in barrens in southeastern Newfoundland (Frey and Gagnon 2016). An intermediate density of 130 urchins m^{-2} (6.5 kg urchins m^{-2}; 50 urchins per straight compartment; 41 urchins per turn compartment) was selected as a halfway point between the two extremes. A high urchin density of 210 urchins m^{-2} (10.5 kg urchins m^{-2}; 80 urchins per compartment) was selected as it is the number of urchins that form approximately one layer of urchins on the bottom of a compartment.

The bottom tier of the raceway was used for a largely exploratory portion of the experiment, involving urchins fed exclusively *Laminaria digitata* kelp instead of the feed (see section 3.2.5). It was performed to explore the differences in gonad growth between urchins fed the feed and urchins fed kelp in the raceway. The bottom tier was divided into one semi-circular turn compartment (~1.6 m^2), and two straight compartments, one before (upstream) and one after (downstream) the turn compartment, each ~0.74 m^2 (~1.3×0.6 m [L×W]; Figure 3.3). Each compartment was divided by 1.3 cm wide separators and contained an urchin density of 13 kg urchins m^{-2} (260 urchins m^{-2}; N=~192 for straight compartments; N=~416 for the turn compartment, N=800 total). Approximately 10 days after starting the experiment, four separators were placed before the start of the upstream straight compartment to increase water flow in the bottom tier to more closely match that in the top two tiers (Figure 3.3).

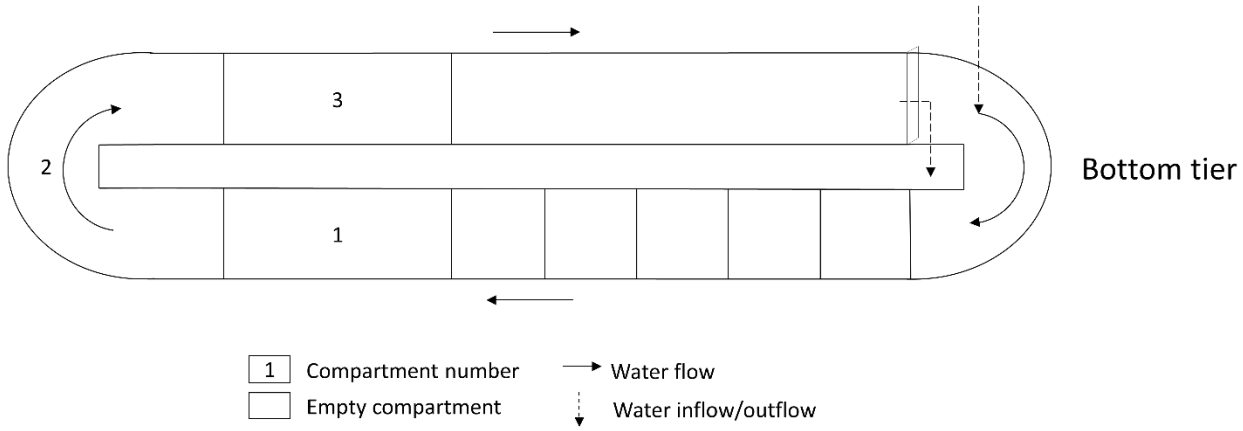

Figure 3.3 Schematic of the bottom tier of the raceway in which urchins were fed kelp (*Laminaria digitata*) for a largely exploratory portion of the experiment. The bottom tier had two straight compartments (1 [upstream] and 3 [downstream]) and one semi-circular turn compartment (2) each with an urchin stocking density of 13 kg urchins m^{-2}. The separators of the five empty compartments before compartment 1 were used to increase water flow in the three main compartments. Water fell into the bottom tier from the end of middle tier, and water flowed out into a drain.

3.2.3 Physiochemical conditions

Water temperature in the raceway was measured every 5 min with two temperature loggers (HOBO Pendant; Onset Computer Corporation) located in the center compartment of the turn in each of the three tiers. Temperature, dissolved oxygen (DO), salinity, and pH were also measured with a YSI Professional Plus (Model Pro 10102030) in all three tiers throughout the experiment at regular intervals (see sections 3.2.4 and 3.2.5).

3.2.4 Feed-fed urchins

Urchins in the top two tiers of the raceway were fed exclusively the formulated feed Urchinomics V9.1.2, hereafter termed the Urchinomics feed (Figure 3.4). Feed pellets contain a proprietary fish-meal and fish-oil based blend of ingredients and are negatively buoyant. A proprietary production process globally licensed to Urchinomics is used to produce the characteristic C-shaped pellets, which increases their water stability to up to seven days in water below ~10°C. This shape also increases the pellets' residence time within specifically designed (SeaNest™) sea urchin holding crates.

Trials with the formulated feed lasted ~72 h, and one trial was carried out per week for seven weeks from 25 January to 14 March, 2018, with 10 days of acclimation from January 15 to 25, 2018 (Figure 3.5). Urchins were not fed during the acclimation phase to standardize hunger levels. The experiment ended after seven trials to limit overlap with the natural peak spawning period of March to May of the green sea urchin in Newfoundland (Himmelman 1978, Keats et al. 1984, Scheibling and Hatcher 2007). Day 1 of each trial involved the set-up of all compartments of the raceway by (1) gently removing the urchins from the compartment and placing them in a

Figure 3.4 C-shaped formulated feed pellets (Urchinomics V9.1.2) provided to urchins during the 7-wk experiment. Feed pellets are negatively buoyant, and maintain physical integrity for up to seven days in water below ~10°C. The curved shape of the feed pellets increases their residence time within specifically designed (SeaNest™) sea urchin holding crates (not shown).

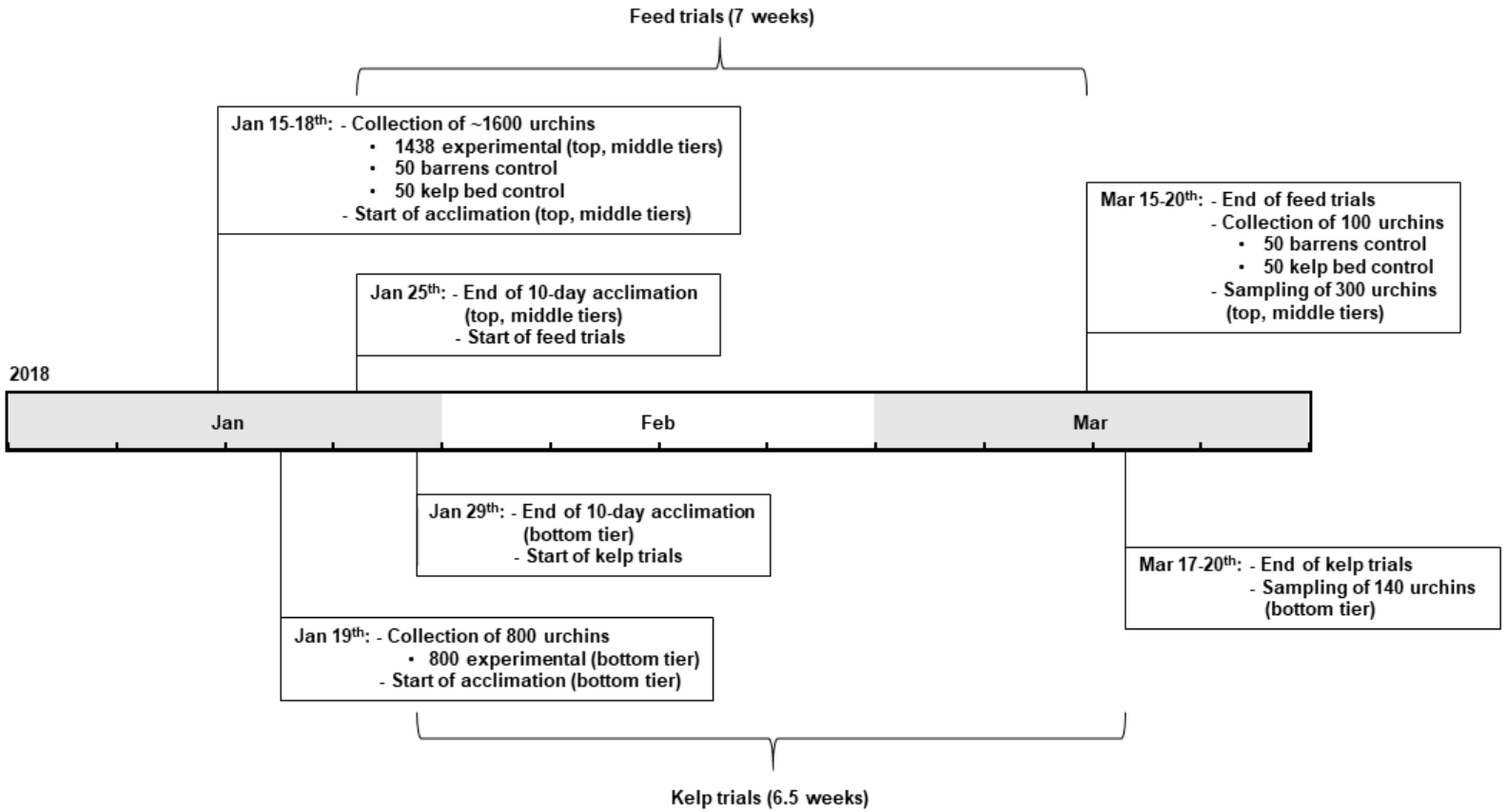

Figure 3.5 Timeline for the raceway experiment including the 7-wk feed trials with green sea urchins (*Strongylocentrotus droebachiensis*) fed the formulated feed in the top and middle tiers, and the 6.5-wk exploratory kelp trials with green sea urchins fed kelp (*Laminaria digitata*) in the bottom tier. Sampling of the 50 barrens and 50 kelp bed urchins (collected both before and after the experiment), the 300 urchins at the end of the feed trials, and the 140 urchins at the end of the kelp trials involved measuring sea urchin body size (test diameter), whole body wet weight, gonad wet weight, and gonadosomatic index (GSI).

seawater-filled plastic bin (~80 L); (2) removing the leftover feed and adding new pre-weighed feed (50 g feed kg^{-1} urchin); and (3) gently removing the urchins from the bin and placing them back into the same compartment, then uniformly spreading the urchins and taking a photo. A photo was taken every 30 min for two hours, then every hour for four hours (nine photos per compartment on Day 1). Photos were taken over the center of each compartment with a GoPro HERO5 Black camera. Physiochemical readings (Table E.2) of temperature, DO, salinity, and pH were taken in the morning (0630), midday (1230), and evening (1830) in the center compartment of each group of three adjacent compartments.

On Day 2 and Day 3, we took a photo of each compartment four times throughout the day (0930, 1130, 1330, and 1530), and physiochemical readings of all compartment groups were recorded throughout the day (1000, 1225, and 1450). On Day 4, a photo of each compartment was taken in the morning (0930) followed by a physiochemical reading of all compartment groups (1000). A total of 10 physiochemical readings per compartment group and a total of 18 photos per compartment were taken during each trial, except in Trial 3 where only 17 photos were taken of compartments 1, 2, and 3 (Figure 3.2) due to a timing error. Day 4 involved (1) counting and removing the urchins from each compartment [to determine urchin movement between compartments] and resetting correct density numbers; (2) removing and drying the feed from each compartment; (3) siphoning out waste (feces, spines) from all compartments; (4) adding new pre-weighed feed (50 g feed kg^{-1} urchin); and (5) replacing the urchins in their new density-randomized compartment within each compartment group (Figure 3.2). Groups of urchins were randomly allocated to each compartment of the same group of three compartments from one trial (week) to the next, ensuring that the same urchins remained in the same density treatment within the same tier and compartment group throughout all trials. Over the following three days, urchins were

allowed to feed on the provided pellets without any manipulation or photographing, until the start of the next trial. Any dead urchins found during daily inspection of the compartments were removed and replaced at the start of the next trial by live urchins kept in the flow-through holding tanks at ambient seawater temperature and fed the formulated feed at 50 g feed kg^{-1} urchin since collection day.

3.2.4.1 Feed consumption

Throughout the experiment, urchins were fed the Urchinomics feed pellets (Figure 3.4): 47.5, 125, and 200 g for the low, intermediate, and high densities, respectively, in the straight compartments, and 102.5 g for the intermediate density in the turns, at the start of each trial. Unconsumed feed was removed from each compartment at the end of each trial. In order for urchins to continuously feed between trials, they were given new feed at 50 g feed kg^{-1} urchin once the unconsumed feed was removed. Unconsumed feed from each compartment was sieved through a 3×3 mm sieve to remove waste matter, dried for approximately 140 h at 50°C, and weighed. Since fresh feed pellets contained water, the following procedure was used to compare fresh and unconsumed feed weights:

Nine samples of feed pellets were weighed out to match the approximate dry weights of the unconsumed feed removed from the low, intermediate, and high densities in the raceway (~20, 50, and 80 g, respectively; N=3 per weight). The pellets were dried for 140 h at 50°C and then weighed again. The percent change in feed weight due to water loss was then calculated with the following equation, as used in Chapter II (section 2.2.3):

$$RWL_w = \frac{IW - FW}{IW} * 100$$

where RWL_w is the relative weight loss due to water loss (in %), IW is the initial (before drying) weight (in g), and FW is the final (after drying) weight (in g). Mean RWL of the nine samples was 5.4% which was then used as a correction factor to calculate the adjusted unconsumed feed dry weight using the following equation, also used in Chapter II (section 2.2.3):

$$ADW = DW + (DW * 0.054)$$

where ADW is the adjusted unconsumed feed dry weight (in g), DW is the unconsumed feed dry weight (in g), and 0.054 is the correction factor.

Because the feed pellets partially dissolved in water, RWL due to dissolution (RWL_d) was calculated. Feed pellets pre-weighed to match the feed weights provided to the low, intermediate, and high densities at the start of each trial (47.5, 125, and 200 g, respectively) were placed in the bottom tier of the raceway in three of the empty compartments (~0.38 m^2 each) for approximately 72 h (the length of one trial). The three feed samples were then collected from each compartment and dried to determine the RWL due to dissolution with the following equation:

$$RWL_d = \frac{IW - FW}{IW} * 100 - RWL_w$$

where RWL_d is the relative weight loss due to dissolution (in %), IW is the initial (before being placed in the water) weight (in g), FW is the final (after drying) weight (in g), and RWL_w is the relative weight loss due to water loss (5.4%). Mean RWL_d of the three samples was found to be 13.7% which was then used as the correction factor to calculate the feed weight lost to dissolution:

$$WL_d = FP * 0.137$$

where WL_d is weight loss due to dissolution (in g), FP is the amount of feed provided at the start of the trial (in g) and 0.137 is the correction factor. The amount of feed consumed was then calculated with the following equation:

$$NFC = FP - WL_d - ADW$$

where NFC is the net feed consumption (in g), FP is the amount of feed provided at the start of the trial (in g), WL_d is the weight lost due to dissolution (in g), and ADW is the adjusted unconsumed feed dry weight (in g). To compare feeding among stocking densities, feed consumption per compartment was then standardized to relative daily feed consumption per compartment with the following formula, similar to that used in Chapter II (section 2.2.3):

$$RFC = \frac{NFC}{UB * t}$$

Where RFC is the relative feed consumption (in g feed kg^{-1} urchin day^{-1}), NFC is the net feed consumption (in g), UB is the urchin biomass in the compartment (0.95, 2.5, or 4.0 kg for the low, intermediate, and high densities, respectively), and t is the number of days over which urchins consumed the feed (~3 days).

3.2.4.2 Aggregation

Images were cropped with *ImageJ* to keep only the compartment of interest and to rescale all photos to a uniform size (Figure 3.6A). Images were then processed in ArcGIS V10.4 (hereafter referred to as GIS) to remove the vertical edges of the compartments from the images (because of artifacts caused by shadows cast by the edges), and to quantify three variables for each image: (1) the number of urchin aggregations in the compartment [urchins in physical contact are considered to be in the same aggregation; an aggregation could be comprised of just one urchin not in contact with any other individuals]; (2) the size of each urchin aggregation, i.e. the surface area covered by the urchin aggregation divided by the surface area covered by all urchin aggregations in the compartment; and (3) the location of urchins within the compartment, i.e. the percentage of urchins in the upstream, middle, and downstream areas in the compartment (Figure 3.6B).

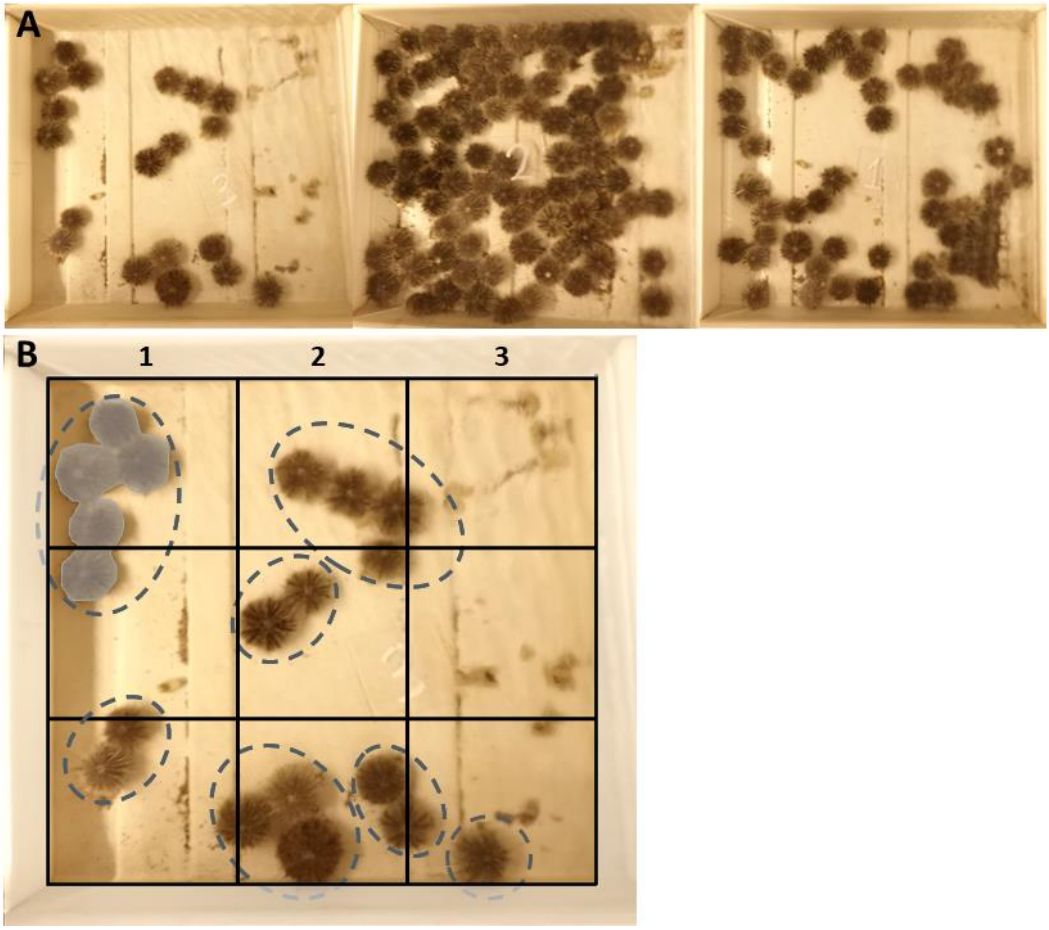

Figure 3.6 Sample images of (A) Position 1 in the raceway with the three urchin stocking densities in (from left to right) compartment 3 (low), 2 (high), and 1 (intermediate), and (B) compartment 3 with the 3×3 grid overlay used for manual visual analysis, the portion outside the 3×3 grid excluded from the GIS analysis (shaded in white/beige) due to edge artifacts, and the variables quantified for each image using the GIS data: urchin aggregations (dotted circles), the area of an urchin aggregation (shaded in grey), and the downstream (column 1), middle (column 2), and upstream (column 3) area separations. Each compartment is 0.7×0.6 m (L×W).

The methods used for processing the images using GIS were based on those used in a study on blue mussels where photos of histological sections were analyzed using GIS to determine the gonad volume fraction (GVF) of the mussels (Murray and Ollerhead 2018). A geographic information system (GIS) was used to analyze images to quantify the areas and distributions occupied by urchins within the compartments. The present study used GIS functionality to analyze the contrast in the images to extract the dark regions representing the urchins and other objects in the tanks. Using area filters, the images were further processed to identify and remove the smaller regions in the tank associated with feed and feces. The remaining urchin-only areas were then analyzed to quantify the number of aggregations, the total area covered as well as the spatial distribution of the areas within the tanks. To validate the automated GIS process (Murray and Ollerhead 2018), a 10% sample (with similar representation for each compartment, trial, time, and stocking density) of all images (302 of the 3021) were manually analyzed in *ImageJ*. Manual analysis was performed by overlaying a grid of 3×3 sections over each image, and counting the number of urchins in each section (Figure 3.6B). The surface area of urchins in each of these sections was also determined with the GIS analysis for comparison with the manual urchin count (see section 3.2.7.2).

Because the GIS analysis involved the removal of the vertical edges from the images, the total number of urchins on the vertical edge of the compartment was manually counted for each image. Urchins were considered on the edge if their entire oral side was on the vertical edge, or if they were tilted so that part of their oral side touched the vertical edge. The percentage of urchins on the edge of each compartment was used for analysis, determined by the total number of urchins on the edge divided by the total number of urchins in each compartment (19, 50, and 80 for the low, intermediate, and high urchin stocking densities, respectively).

3.2.5 Kelp-fed urchins

Exploratory trials with kelp were ran simultaneously with the formulated feed trials, lasting 6.5 weeks from January 29 to March 16, 2018, with approximately 10 days of acclimation before the start of trials from January 19 to 29, 2018 (Figure 3.5). Urchins were not fed during the acclimation phase to standardize hunger levels. Kelp (*Laminaria digitata*) was collected from BCC before the start of the trials and throughout the experiment (approximately every 2 weeks), and was kept in ambient flow-through holding tanks until use. On the first day of the kelp trials, urchins and separators in the bottom tier were arranged in the compartments (see section 3.2.2; Figure 3.3) and were then completely covered with kelp. Before providing the kelp to the urchins, it was manually processed by cutting off the stipes and removing portions with epibionts and epiphytes, and portions undergoing decomposition. To ensure general consistency, kelp individuals were cut into blade groups containing ~1-4 blades totalling a width of ~5-10 cm and a length of ~20-100 cm. Processed blade groups were laid over the urchins in layers (parallel to the length of the raceway) so that all urchins were completely covered with a similar amount of kelp. Urchins were fed approximately 70 kg of kelp (wet weight) over the 6.5 weeks, and new kelp was added 0-2 times per week to ensure continuous access to kelp. Any urchin mortalities found during daily inspection were removed.

On the first day of the kelp trials, physiochemical readings were taken in compartment 2 of the bottom tier (Figure 3.3). Afterwards, physiochemical readings (Table E.2) were recorded twice a week (Wednesday at 1300, Saturday at 1100), in each of the three compartments (Figure 3.3) as well as in the empty compartment directly before the first compartment (b1) and directly after the third compartment (a3). For the last two weeks, 10 additional physiochemical readings per week were taken in b1 directly after the completion of the readings of the top two feed tiers

(see section 3.2.4). Physiochemical readings totalled 32 for b1, 13 for compartments 1, 3, and a3, and 14 for compartment 2. Waste (feces and spines) was siphoned out of the bottom tier twice a week.

3.2.6 Gonad yield

We used the gonadosomatic index (GSI) to compare gonad yields, calculated using the commonly-used formula:

$$GSI\ (\%) = \frac{Gonad\ wet\ weight\ (g)}{Whole\ urchin\ wet\ weight\ (g)} * 100$$

The GSI of each urchin was determined for the 50 kelp bed urchins and 50 barrens urchins collected from BCC at the beginning and end of the experiment (used as controls), 240 feed-fed urchins from the straight segments and 60 from the turns of the raceway (N=10 per compartment) at the end of the experiment, and 140 kelp-fed urchins from the bottom tier of the raceway at the end of the experiment (Figure 3.5). Due to a measurement error in the bottom tier, one urchin was excluded from analysis (N=40 for compartments 1 and 3; N=59 for compartment 2; N=139 total). In addition to the GSI, the test diameter of each sampled urchin was also measured using an electronic caliper.

3.2.7 Statistical analysis

3.2.7.1 Feed consumption

Differences in relative feed consumption (RFC, N=168) were compared using a four-way ANOVA with the fixed factors of Density (the three urchin stocking densities: 50, 130, and 210 urchins m^{-2}), Position (four positions: 1-4; see Figure 3.2 for spatial layout), and Tier (top and middle tiers), and the fixed covariate of Trial (seven trials). Once it was found that the Trial

covariate was significant, regression analysis was used to determine the strength of the relationship between Trial and RFC. To compare overall RFC between raceway straight segments (compartments 1-24) and turns (compartments e1-e6; see Figure 3.2 for spatial layout), a Welch's two sample t-test was used.

3.2.7.2 Aggregation

The surface area of urchins determined using the GIS method and the number of urchins counted manually, in each of the nine sections (N=2718) and in each of the three streams (upstream, middle, and downstream; N=906) of the 302 sample images were compared using a simple linear regression analysis. Strong, significant coefficients of determination (p<0.001, R^2>0.90) were found between the GIS and manual results (Figure F.1). The strong, highly significant relationships between the GIS and manual results of urchins found in each of the nine sections and in each stream demonstrates that the GIS method accurately detects urchins and their location within each compartment. Therefore, the GIS results are accurate and acceptable measures for use in further analyses.

The changes in the number of urchin aggregations and the size of aggregations (mean percentage of urchins in each aggregation) were plotted and visually assessed to determine general trends over time and to determine after how many hours from initial placement of urchins in the compartment did urchin aggregation (number and size) stabilize. Aggregations changed rapidly within the first couple of hours, and were found to stabilize after ~20 h from initial placement (Figure F.2). Therefore, the number of aggregations, the mean percentage of urchins found in each aggregation, the percentage of urchins found in the downstream, middle, and upstream areas of the compartment, and the percentage of urchins found on the compartment edges were averaged from

time 10 (~20 h from initial placement) to time 18 (~72 h from initial placement) (N=9 photos per compartment per trial) for use in further analyses.

Differences in the number of urchin aggregations (N=168), the mean percentage in each aggregation (N=168), and the percentage of urchins on the edge of the compartments (N=168) were each tested using a four-way ANOVA with the fixed factors of Density (the three urchin stocking densities: 50, 130, and 210 urchins m^{-2}), Position (four positions: 1-4; see Figure 3.2 for spatial layout), and Tier (top and middle tiers), and the random factor Trial (trials 1-7). Differences in the location of urchins within each compartment (N=504) were tested using a five-way ANOVA with the same factors as above along with an additional fixed factor of Area within each compartment (downstream, middle, and upstream areas; see Figure 3.6B for area layout).

3.2.7.3 Gonad yield

Differences in mean gonadosomatic index (GSI) of the feed-fed urchins from the raceway straight segments (N=24) were tested using a four-way ANOVA with the fixed factors of Density (the three urchin stocking densities: 50, 130, and 210 urchins m^{-2}), Location (1 and 2), and Tier (top and middle tiers), and the random factor of block (North vs. South end of the raceway; see Figure 3.2 for spatial layout). Differences in the GSI of the seven urchin groupings (top and middle tier straight segments [feed-fed, N=240] and turns [feed-fed, N=60], raceway bottom tier [kelp-fed, N=139], barrens [field control] onset [N=50] and end [N=50], and kelp bed [field control] onset [N=50] and end [N=50]) were tested using a one-way ANOVA with the fixed factor of urchin grouping.

We also calculated the percent change in GSI (ΔGSI) between the beginning and end of the experiment for each urchin, using the following formula:

$$\Delta GSI\,(\%) = \left[\frac{GSI_E - GSI_B}{GSI_B}\right] * 100$$

where ΔGSI is the percent change in GSI, GSI_E is the GSI at the end of the experiment. GSI_B for the raceway urchins, and the barrens urchins collected at the end of the experiment is the mean GSI of the 50 urchins collected from the barrens in BCC before the start of the experiment. GSI_B for the kelp bed urchins collected at the end of the experiment is the mean GSI of the 50 urchins collected from the kelp bed in BCC before the start of the experiment. Differences in ΔGSI were compared using ANOVAs identical to the two described above for the GSI comparison, except the comparison of the seven urchin groupings now only has five groupings (top and middle tier straight segments [feed-fed, N=240] and turns [feed-fed, N=60], raceway bottom tier [kelp-fed, N=139], barrens at experiment end [field control, N=50], and kelp bed at experiment end [field control] onset, N=50]) since the barrens GSI and kelp bed GSI at experiment onset do not have a ΔGSI value.

In all ANOVAs and simple linear regression analysis, we verified homogeneity of variance and normality of residuals by examining the distribution of the residuals and the normal probability plot of the residuals, respectively (Snedecor and Cochran 1994). These criteria were met in all analyses applied to the raw data. In all ANOVAs, the expected mean squares (EMS), and corresponding F ratios and *p*-values, were calculated according to procedures for mixed models set by Quinn and Keough (2002). Differences among levels within a factor were determined with Tukey HSD multiple comparison tests for the ANOVAs, based on least-square means (Quinn and Keough 2002, Sokal and Rohlf 2012). We used R 3.4.1 (R Core Team 2018) and a significance level of 0.05 for all analyses.

3.3 RESULTS

3.3.1 Water physiochemical conditions

Water temperature remained fairly consistent throughout the experiment, except for a few drops in temperature (Table E.1; Figure E.1) due to system malfunctions, however no marked changes in urchin behaviour were observed. Throughout the seven weeks of the feed trials, water temperature measured by the temperature loggers averaged 6.1, 6.3, and 6.3°C in the top (feed), middle (feed), and bottom (kelp) tiers, respectively (Table E.1; Figure E.1). These averages demonstrate a slight increase in temperature as water flowed from one tier to the next. Temperature increased as water flowed downstream within tiers, with a 0.05-0.15°C increase from the first to the last compartment within each tier (Table E.2). Conversely, dissolved oxygen (DO) decreased from one tier to the next and as water flowed downstream within each tier, averaging 121.7, 105.2, and 97.2% in the top, middle, and bottom tiers, respectively, and with a 3.4-7.0% decrease from the first to the last compartment within each tier (Table E.2; Figure E.2). Overall, DO remained fairly consistent from one week to the next (Figure E.2). Salinity remained consistent throughout the experiment and from one tier to the next, averaging 31.4 ppt in all tiers, and there was a slight decrease in pH from one tier to the next, averaging 8.3, 8.3, and 8.2 in the top, middle, and bottom tiers, respectively (Table E.2).

3.3.2 Urchin bodily characteristics, mortality, and movement between compartments

In the top and middle (feed) tiers of the raceway, the test diameter of urchins reached an average of 48.3 mm, only increasing by up to ~2%, however body weight increased by up to ~15% (averaging 55.3 g) because of the increase in gonad weight of up to ~544% (Table G.1). In the bottom (kelp) tier of the raceway, test diameter of urchins reached an average of 49.3 mm, only

increasing by ~3%, however body weight increased by ~18% (averaging 59.1 g) because of the increase in gonad weight of ~283% (Table G.1). There was low mortality in the raceway, with a total of six (0.4%) and 36 (4.5%) mortalities in the top two (feed) tiers and bottom (kelp) tier, respectively. Overall, the mean number of urchins that moved into or out of each compartment in the top two (feed) tiers per trial was <1 urchin.

3.3.3 Feed consumption

Relative feed consumption (RFC) differed between the top and middle (feed) tiers among positions (see Figure 3.2 for spatial layout), however was similar among urchin stocking densities (Table H.1). RFC was lowest in Positions 3 and 4 of the middle tier, averaging 6.1±0.2 [SE] g feed kg^{-1} urchin day^{-1}, and similarly highest in all other positions, averaging 7.1±0.2 g feed kg^{-1} urchin day^{-1} (Figure 3.7). Position 3 of the middle tier was lower than all other positions (by at least 13%), and Position 4 of the middle tier was lower than all other positions except for Positions 1 and 4 of the top tier (Figure 3.7).

RFC also varied over time from the first to the seventh (last) trial (Table H.1). RFC increased (by ~33%) from the 1^{st} to the 4^{th} trial, plateauing at 7.6±0.1 g feed kg^{-1} urchin day^{-1}), and then decreased (by ~13%) from the fifth to the last trial (Figure 3.8; Table G.2). By comparing the pooled RFC values from the 24 straight segment compartments (6.8±0.1 g feed kg^{-1} urchin day^{-1}) with the pooled RFC values from the six turn compartments (7.3±0.1 g feed kg^{-1} urchin day^{-1}) across all trials, the turns had a ~7% higher RFC than the straight segments (p<0.01, Table G.2). The turns had a similar feeding pattern over time to the straight segments, with RFC increasing, plateauing, and decreasing over the seven trials (Table G.2).

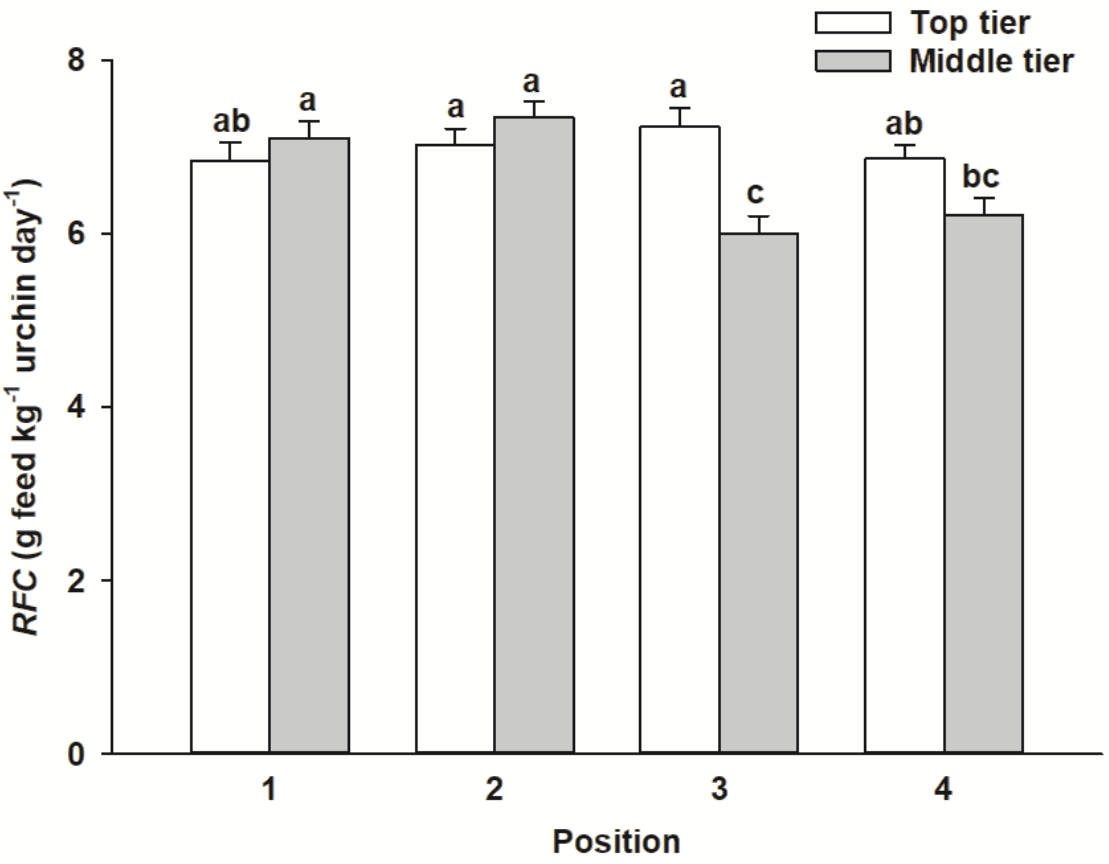

Figure 3.7 Mean (±SE) relative feed consumption (RFC) of green sea urchins fed the formulated feed over seven trials in each of the four positions (1-4; see Figure 3.2 for spatial layout) in each of the two tiers (top and middle) of the raceway. Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$, n=21 for each bar).

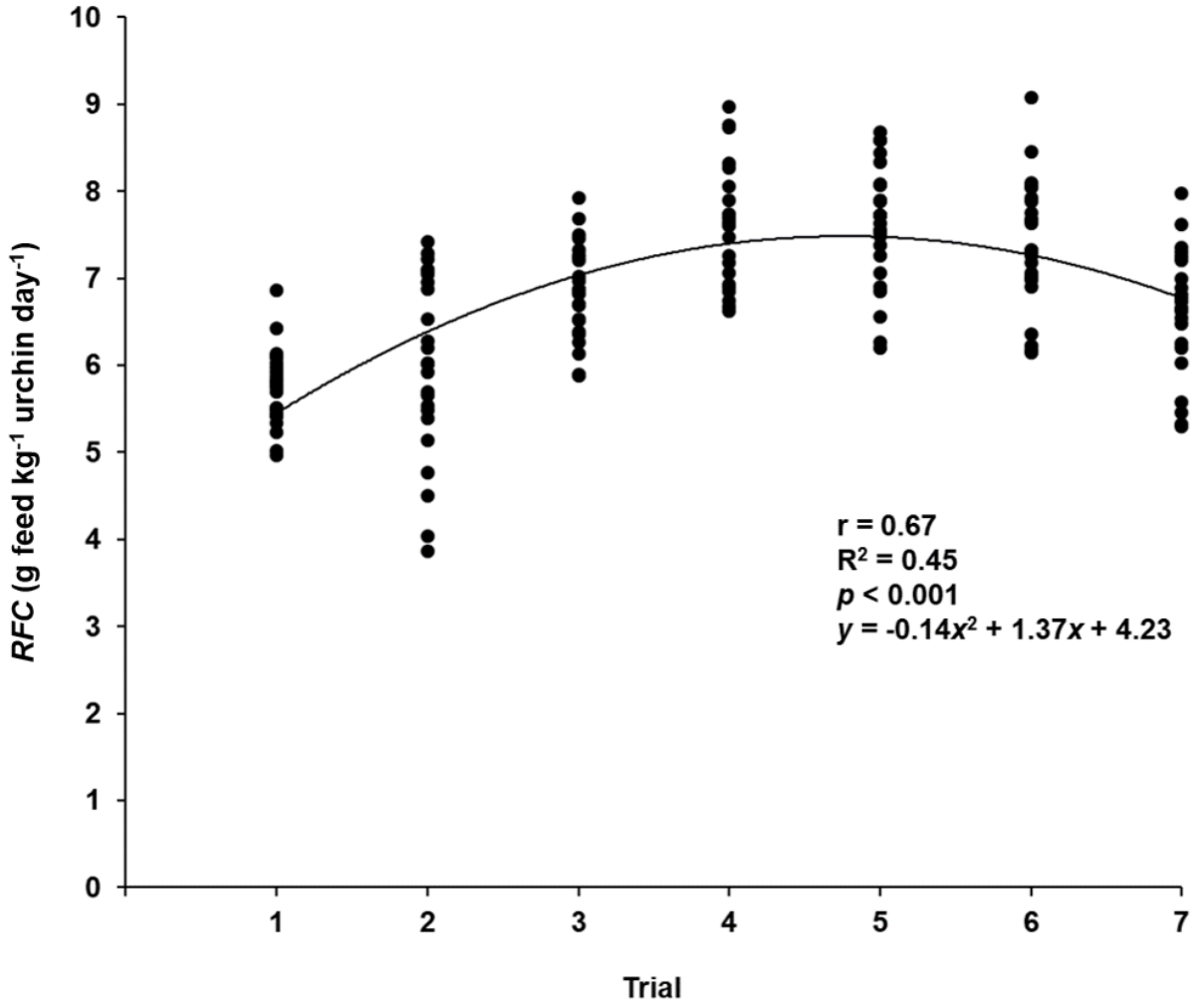

Figure 3.8 Relative feed consumption (RFC) of green sea urchins fed the formulated feed in the raceway (top and middle tiers) over the seven trials (n=24 per trial).

3.3.4 Aggregation

High, significant coefficients of determination were found between the surface area of urchins (determined using the GIS method) and the number of urchins (counted manually) in each of the nine sections (p=0, R^2=0.91) and in each of the three streams (p=0, R^2=0.93) of the 302 sample images (Figure F.1). The strong, highly significant relationship between GIS and manual results of urchins found in each of the nine sections and in each stream demonstrates that the GIS method accurately detects urchins and their location within each compartment. Therefore, the GIS results are accurate and acceptable measures for use in further analyses.

3.3.4.1 Number of aggregations

The initial placement of individual urchins (one urchin per aggregation) was 19, 50, and 80 aggregations for the low, intermediate, and high urchin stocking densities, respectively. The mean number of urchin aggregations was ~66, 88, and 95% lower after stabilization (after 20h) compared to the initial placement of urchins for the low, intermediate, and high densities, respectively (Figure F.2). The number of aggregations of urchins in each compartment differed among urchin stocking densities and raceway positions (Table H.2). Urchins in the high density formed the least number of aggregations (by at least 34%), averaging 3.9±0.1 aggregations, compared to urchins in the intermediate and low densities with a statistically similar number of aggregations (~6.1±0.1; Figure 3.9). The number of urchin aggregations was statistically similar and lowest (by ~26%) in Positions 1 and 2 (~4.6±0.1 aggregations) compared to in Positions 3 and 4, which were also statistically similar (~6.2±0.2 aggregations) (Figure 3.10).

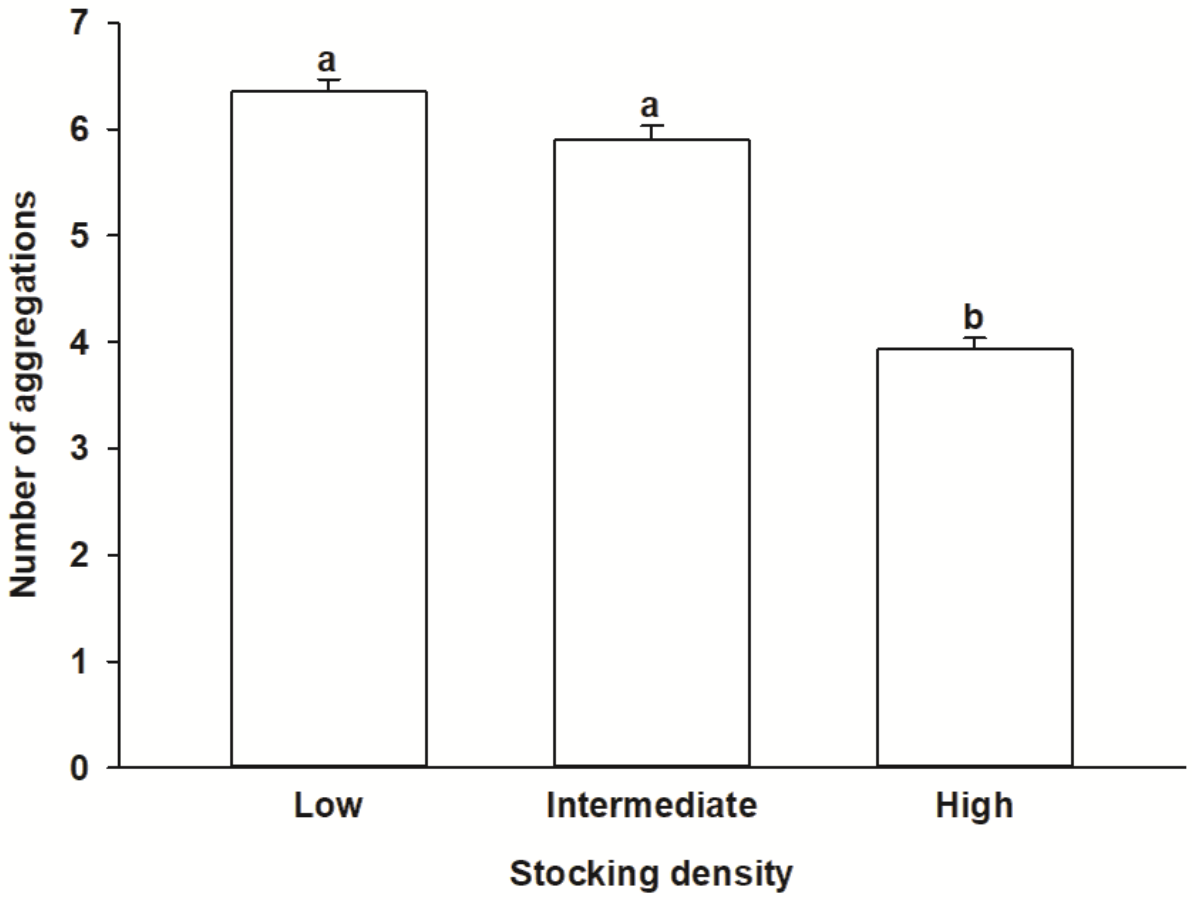

Figure 3.9 Mean (+SE) number of aggregations of green sea urchins in each compartment after 20hr from initial placement of the urchins in the low, intermediate, and high urchin stocking densities. Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$, n=56 for each bar).

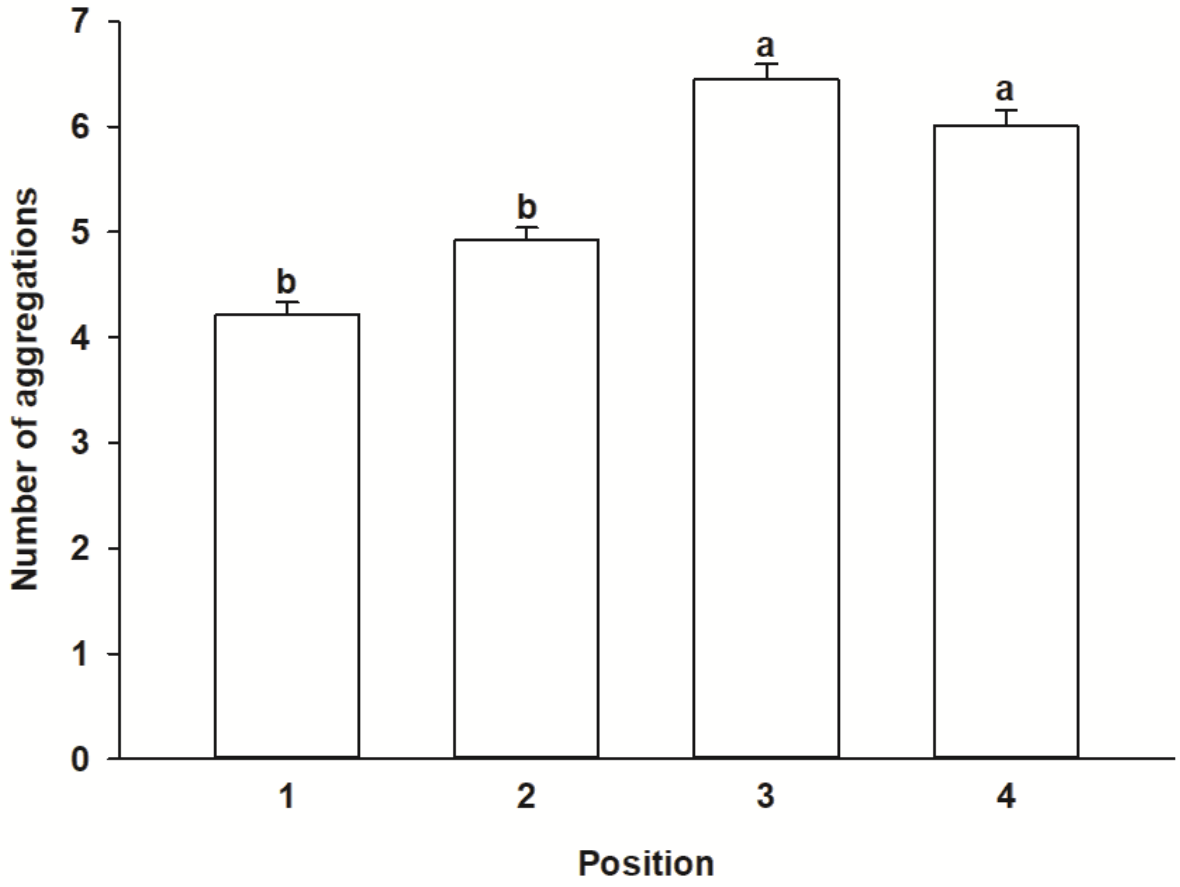

Figure 3.10 Mean (+SE) number of aggregations of green sea urchins in each compartment after 20hr from initial placement of the urchins in each raceway position (1-4; see Figure 3.2 for spatial layout). Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$, n=42 for each bar).

3.3.4.2 Size of aggregations

The mean size of urchin aggregations (mean percentage of urchins found in each aggregation) after stabilization (after 20h) was 18.7, 21.5, and 37.2%, ~3, 10, and 28 times higher compared to the initial individual placement of urchins (one urchin per aggregation; 5.3, 2.0, and 1.3%), for the low, intermediate, and high urchin stocking densities, respectively (Figure F.2). The mean size of each aggregation differed among urchin the three stocking densities among raceway positions (Table H.3). In the high density, the percentage of urchins per aggregation was highest in Position 1 (by at least 50%) at 54.2±2.8% compared to in Positions 2, 3, and 4 which were all statistically similar (~31.5±1.9%; Figure 3.11). In the intermediate and low densities, the percentage per aggregation was similar among all positions, averaging 21.5±3.9% and 18.7±0.8%, respectively (Figure 3.11). The percentage per aggregation was highest in the high density compared to the intermediate and low densities (by at least 82%) in Positions 1 and 4 (Figure 3.11). In Position 2, the high density was statistically similar to the intermediate density but higher than the low density, and in Position 3 the percentages were similar among all three densities (Figure 3.11). Percentages in the intermediate and low densities were statistically similar in each position (Figure 3.11).

3.3.4.3 Stream preference

Urchins were initially placed uniformly in each compartment (~33.3% in the downstream, middle, and upstream areas). After stabilization (after 20h), the percentage of urchins increased (by ~21, 21, and 14%) in the downstream area and decreased in the middle area (by ~32, 12, and 5%), for the low, intermediate, and high urchin stocking densities, respectively (Figure F.3). In the

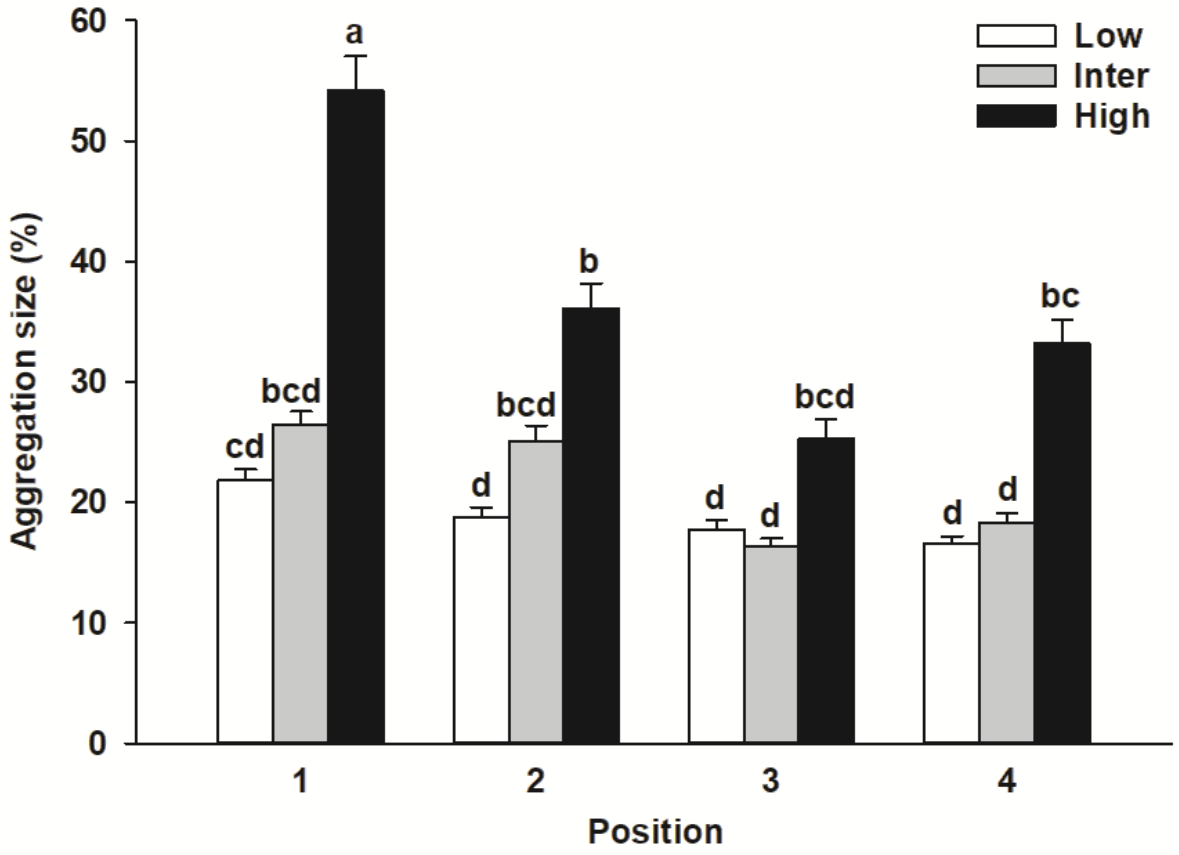

Figure 3.11 Mean (+SE) percentage of green sea urchins per aggregation after 20hr from initial placement of the urchins, in each raceway position (1-4; see Figure 3.2 for spatial layout). Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$, n=14 for each bar).

upstream area, the percentage of urchins increased by ~11% in the low density, and decreased by ~8 and 9% in the intermediate and high densities, respectively (Figure F.3).

The percentage of urchins found in each compartment area (downstream, middle, and upstream) differed among the three stocking densities among the four raceway positions, among compartment areas (Table H.4). Of the downstream, middle, and upstream areas of the compartments, urchins in the low density preferred the downstream area in Position 1 and 2, and the upstream area in Position 3 (Figure 3.12). In Position 4, urchins preferred the upstream area compared to the middle, but there was no difference in preference was between the upstream and downstream areas (Figure 3.12). In the intermediate density, urchins preferred the downstream area in Position 2, but showed no preference in the other three positions (Figure 3.12). In the high density, urchins preferred the downstream area in Position 1, but showed no preference in the other positions (Figure 3.12). The percentage of urchins found in each area of the compartment did not differ among densities for any position (Figure 3.12).

The percentage of urchins found in each compartment area also differed among the three stocking densities between the two tiers, among compartment areas (Table H.4). Of the downstream, middle, and upstream areas, in the low density, urchins preferred the downstream area in the top tier whereas there was no difference in preference between the downstream and upstream areas in the middle tier, but both were preferred over the middle area (Figure 3.13). In the intermediate density, urchins preferred the downstream area in the top tier, but showed no preference among any of the areas in the middle tier (Figure 3.13). In the high density, urchins showed no preference among any of the areas in either tier (Figure 3.13). The percentage of urchins found in each area of the compartment did not differ among densities for the top tier, but in the

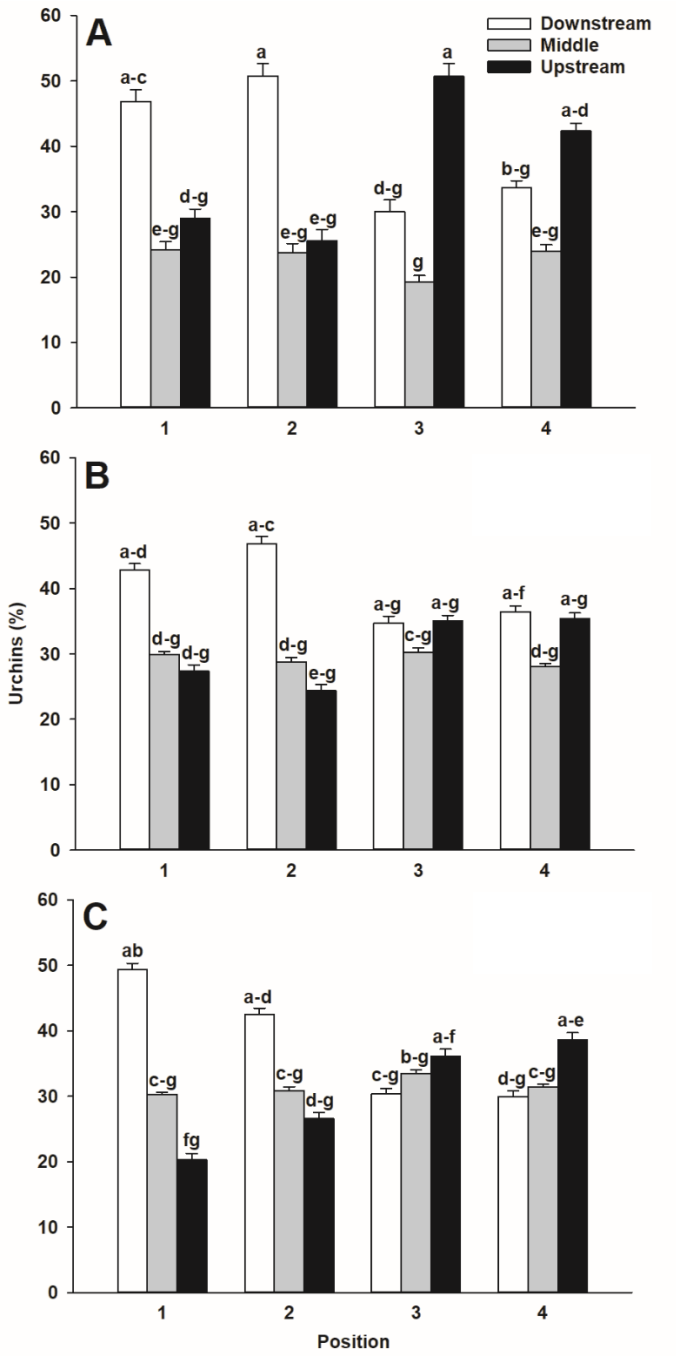

Figure 3.12 Mean (+SE) percentage of green sea urchins found in the downstream, middle, and upstream areas within the compartments after 20hr from initial placement of the urchins in each raceway position (1-4; see Figure 3.2 for spatial layout) in the low (A), intermediate (B), and high (C) urchin stocking densities. Bars not sharing the same letter within or among panels are statistically different (LS means tests, $p<0.05$, n=14 for each bar).

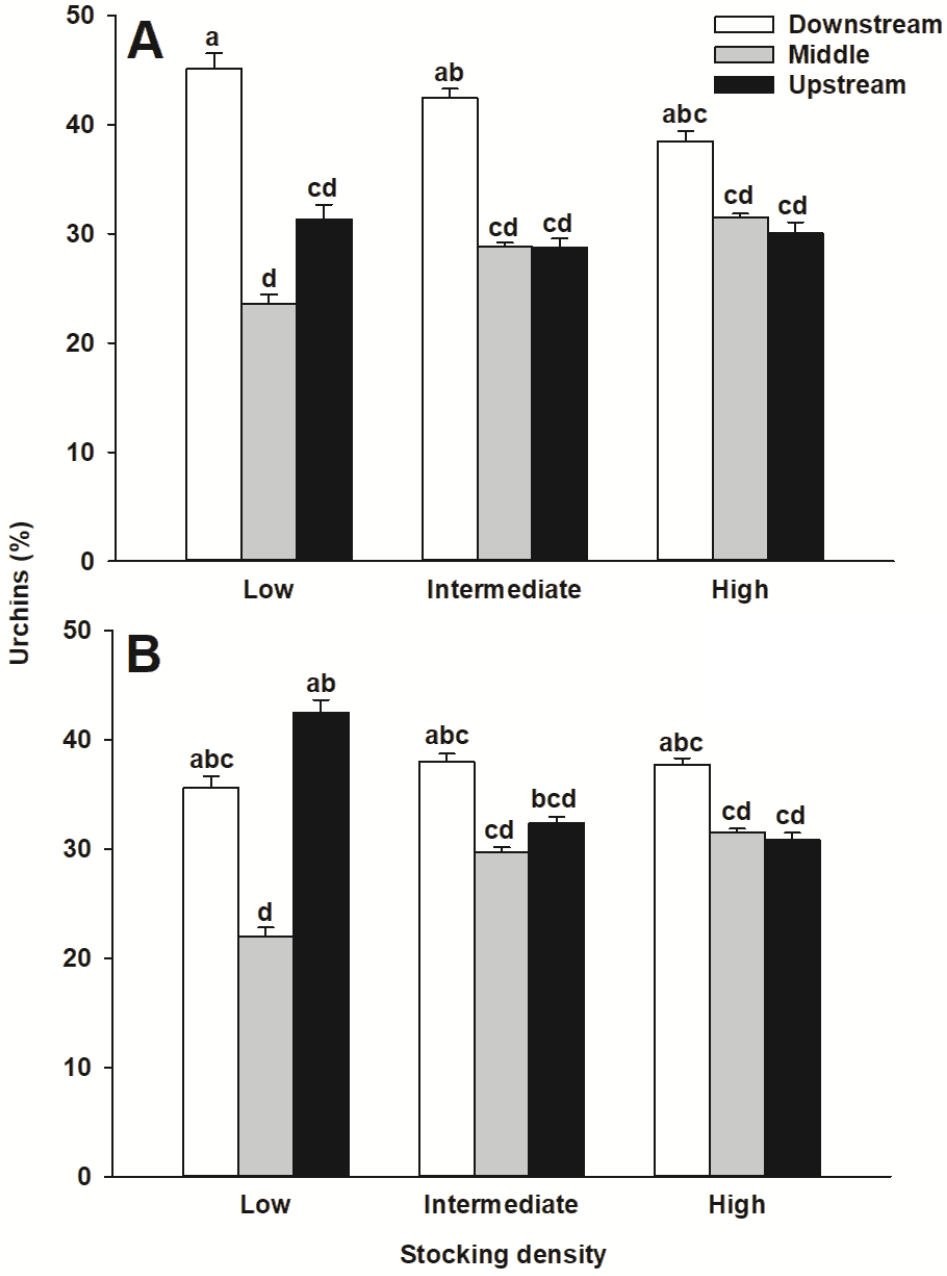

Figure 3.13 Mean (+SE) percentage of green sea urchins found in the downstream, middle, and upstream areas in each compartment after 20hr from initial placement of the urchins in each stocking density (low, intermediate, and high) in the top (A) and middle (B) tiers. Bars not sharing the same letter within or among panels are statistically different (LS means tests, $p<0.05$, n=28 for each bar).

middle tier, a higher percentage of urchins was found in the upstream area in the low density compared to the high density (Figure 3.13).

The percentage of urchins found in each compartment area also differed among the four raceway positions between tiers, among compartment areas (Table H.4). Of the downstream, middle, and upstream areas, in the top tier urchins preferred the downstream area in Position 1 and 2, showed a similar preference between the downstream and upstream areas in Position 3, with a higher preference for the upstream compared to the middle area, and showed no difference in preference among areas in Position 4 (Figure 3.14). In the middle tier, urchins showed a similar preference between the downstream and upstream areas in Positions 1 and 2, with a higher preference for the downstream compared to the middle area, and showed no difference in preference among areas in Positions 3 and 4 (Figure 3.14). The percentage of urchins found in each area of the compartment did not differ among tiers for any position (Figure 3.14).

3.3.4.4 Compartment edge

The mean percentage of urchins found on the edge of the compartments increased from 0% (at initial placement of all urchins on the flat surface of each compartment) to 33.5, 32.2, and 29.5% after stabilization (after 20h) for the low, intermediate, and high urchin stocking densities, respectively (Figure F.4). The percentage of urchins found on the edge of the compartments differed among the three stocking densities among raceway positions, between tiers (Table H.5). In the top tier, urchins in the low density had the similarly highest percentage of edge urchins in Position 3 and 4 (~41.9±2.6%) and the similarly lowest percentage in Positions 1 and 2 (~28.5±2.2%), with similar percentages in Positions 2 and 3 (Figure 3.15). In the middle tier,

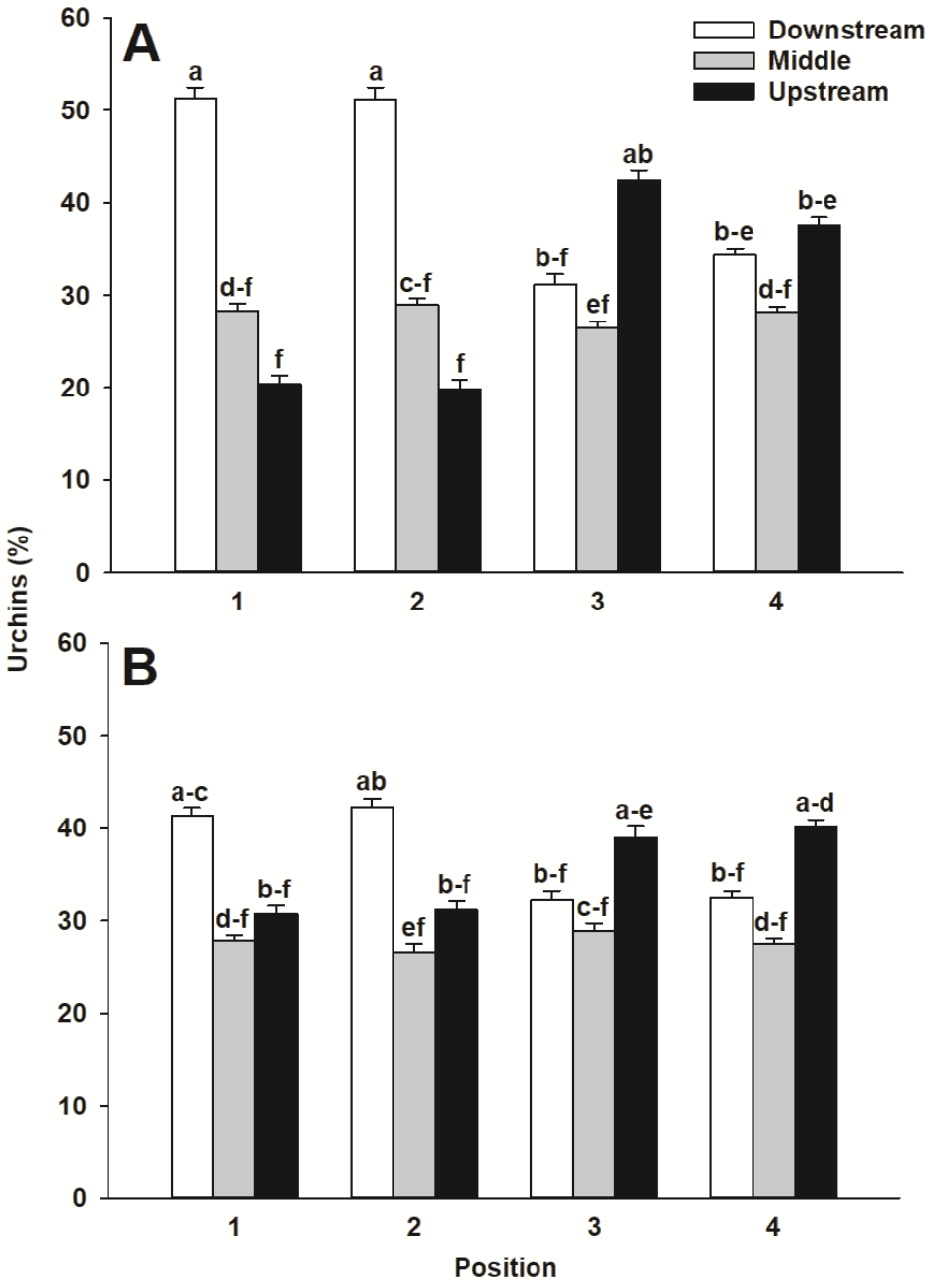

Figure 3.14 Mean (+SE) percentage of green sea urchins found in the downstream, middle, and upstream areas in each compartment after 20 h from initial placement of the urchins in each raceway position (1-4; see Figure 3.2 for spatial layout) in the top (A) and middle (B) tiers. Bars not sharing the same letter within or among panels are statistically different (LS means tests, $p<0.05$, n=21 for each bar).

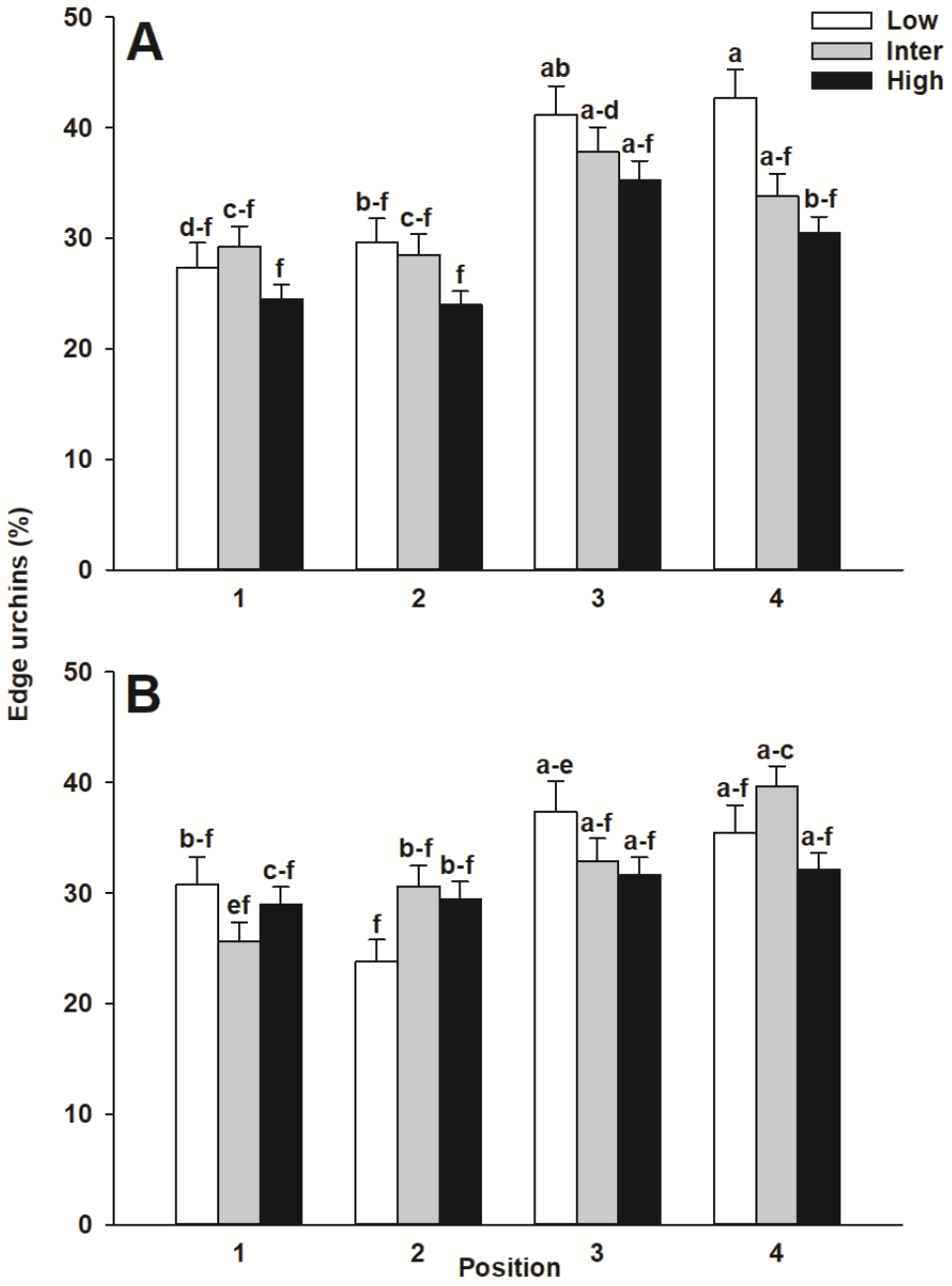

Figure 3.15 Mean (+SE) percentage of green sea urchins on the edge of each compartment after 20hr from initial placement of the urchins in each stocking density (low, intermediate, and high) and raceway position (1-4; see Figure 3.2 for spatial layout) in the top (A) and middle (B) tiers. Bars not sharing the same letter within or among panels are statistically different (LS means tests, $p<0.05$, n=7 for each bar).

urchins in the low density had similar percentages among all positions except for a ~57% higher percentage in Position 3 (37.3±2.8%) compared to Position 2 (Figure 3.15). Urchins in the intermediate density had similar percentages in all positions in the top tier (~32.3±2.0%) whereas in the middle tier, all positions were similar except for a ~55% higher percentage in Position 4 (39.7±1.8%) compared to Position 1 (Figure 3.15). In both the top and middle tiers, urchins in the high density had similar percentages of edge urchins among all positions, averaging 28.6±1.4% and 30.5±1.6% for the top and middle tiers, respectively; Figure 3.15). Within each density, percentages of edge urchins were statistically similar between tiers for each position (Figure 3.15). Percentages were also similar among densities within each position except in Position 4 of the top tier, with a ~40% higher percentage of edge urchins in the low density compared to the high density (Figure 3.15).

3.3.5 Gonad yield

Gonadosomatic index (GSI) and percent change in GSI (ΔGSI) of the feed-fed urchins in the top two tiers of the raceway were similar among stocking densities, locations, and tiers (Table H.6 [GSI] and H.7 [ΔGSI]), with an average GSI of 20.8±0.2% and an average ΔGSI of 474.4±6.0% (Table G.1). GSI differed among the seven urchin groupings (Table H.8). GSI was similar in the raceway straight segments and turns of the top two (feed) tiers, averaging 20.5±0.4% which was significantly higher than all other groupings, including the bottom (kelp) tier of the raceway (12.0±0.3%) by ~71% (Figure 3.16; Table G.1). At experiment onset, GSI of the field urchins was 3.6±0.3% in the barrens (lowest of all groupings) and 16.5±0.8% in the kelp bed (second highest after the raceway feed-fed urchins; Figure 3.16; Table G.1). GSI was similar between the barrens and kelp bed field urchins at the end of the experiment (averaging 9.2±0.7%)

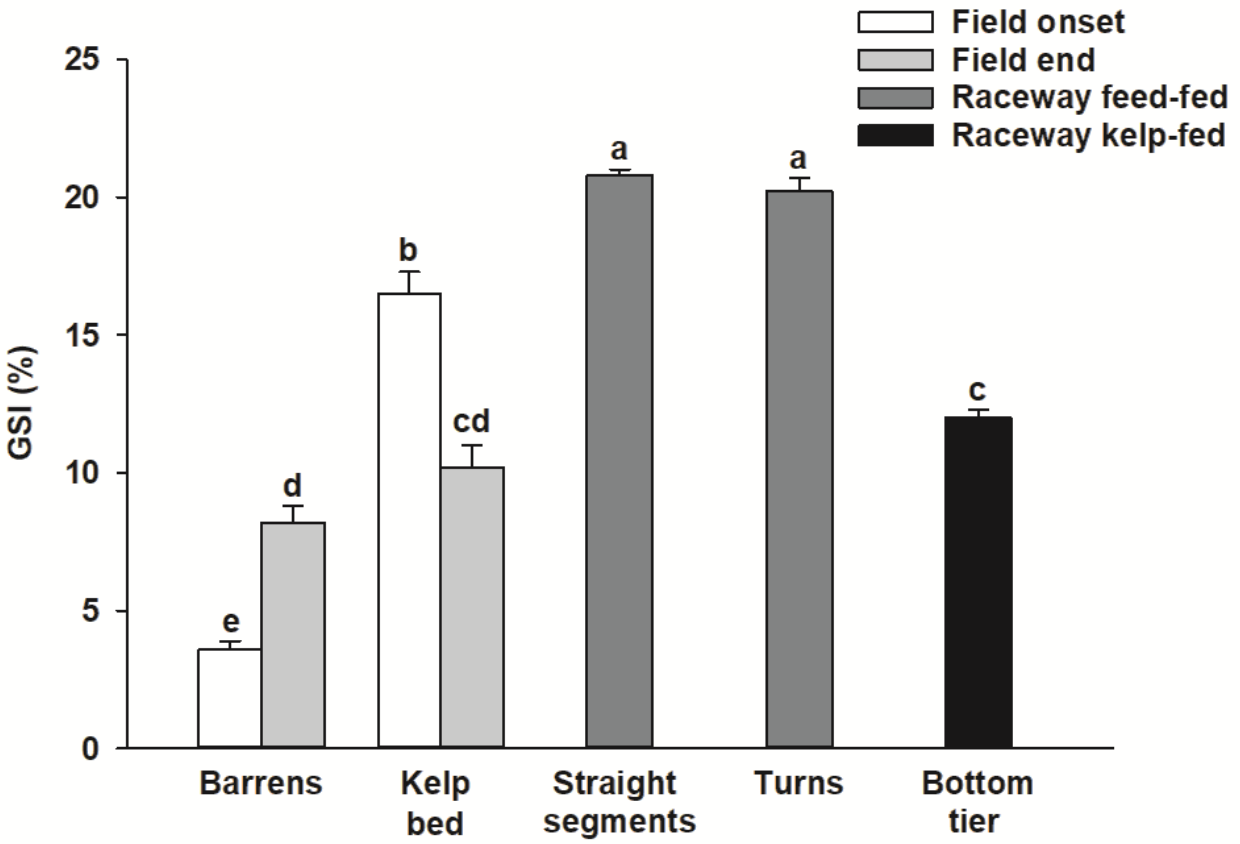

Figure 3.16 Mean (±SE) gonadosomatic index (GSI) of green sea urchins from the field barrens and kelp bed at the onset and end of the experiment (n=50 per bar), from the straight segments (n=240) and turns (n=60) of the top two (feed) tiers of the raceway after the 7-wk feed trials, and from the bottom (kelp) tier after the 6.5-wk kelp trials (n=139). The GSI of urchins from the barrens at onset is also the GSI of the raceway feed- and kelp-fed urchins at onset. Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$). See Figures 3.2 and 3.3 for spatial layouts of the raceway.

as well as between the kelp bed field urchins at the end of the experiment and the bottom (kelp) tier of the raceway (Figure 3.16; Table G.1).

The ΔGSI differed among the five urchin groupings (Table H.9). Similar to GSI, ΔGSI was similar in the raceway straight segments and turns of the top two (feed) tiers, averaging 466.3±9.6%, which was significantly higher than all other groupings, and double that of the bottom (kelp) tier of the raceway (231.1±8.3%) (Figure 3.17; Table G.1). The ΔGSI of the field urchins was 127.1±16.5% from the barrens which was significantly higher than from the kelp bed (-37.9 ±4.8%) as GSI increased from experiment onset in the barrens, but decreased in the kelp bed (Figure 3.17; Table G.1). The ΔGSI of the bottom (kelp) tier was significantly higher than both the barrens and kelp bed field urchins, by at least 82% (Figure 3.17; Table G.1).

3.4 DISCUSSION

3.4.1 Feed consumption

Despite expectations of dense aggregations in the high density treatment restricting feed intake, there were no differences in RFC among densities, averaging 6.8g feed kg^{-1} urchin day^{-1}. Results are comparable to a previous study in Norway on green sea urchins fed a prepared diet for eight weeks in a single-tiered raceway at ambient temperatures (4.5 to 6.5°C) from mid-November to early-January, resulting in a feed intake of ~5.3 g feed kg^{-1} urchin day^{-1} with no differences among three stocking densities (2.5, 3.7, and 7.3 kg urchins m^{-2}; Christiansen and Siikavuopio 2007). The lowest RFC was found in Positions 3 and 4 of the middle tier of the raceway which had the lowest dissolved oxygen (DO) levels of all positions. DO was likely reduced in these positions due to oxygen consumption by urchins and by bacterial degradation of organic waste in

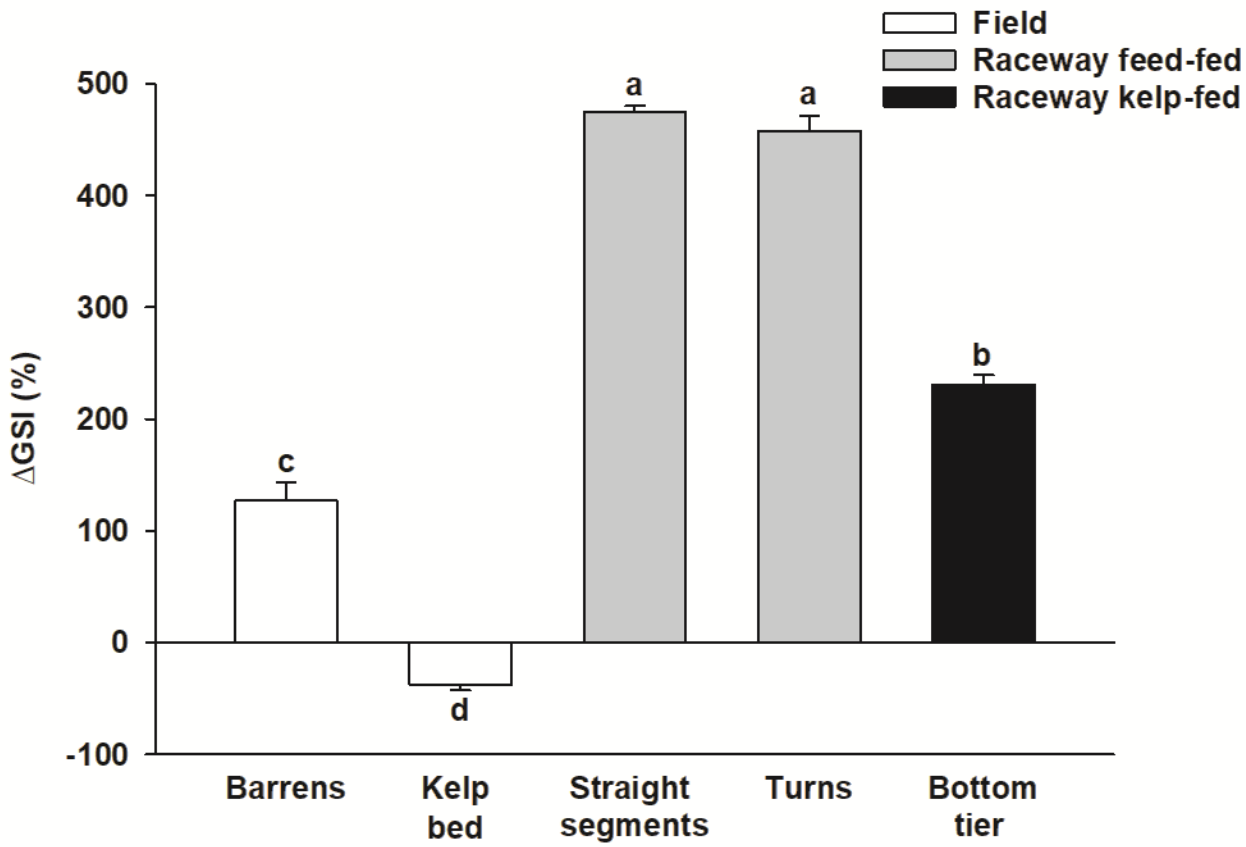

Figure 3.17 Mean (±SE) change in gonadosomatic index (ΔGSI) of green sea urchins from the field barrens and kelp bed at the end of the experiment (n=50 per bar), from the straight segments (n=240) and turns (n=60) of the top two (feed) tiers of the raceway after the 7-wk feed trials, and from the bottom (kelp) tier after the 6.5-wk kelp trials (n=139). Bars not sharing the same letter are statistically different (LS means tests, $p<0.05$). See Figure 3.2 and 3.3 for spatial layouts of the raceway.

all preceding compartments in the top and middle tiers. Low oxygen levels have been found to reduce feed consumption in green sea urchins (Siikavuopio et al. 2007d), therefore the lower DO in these positions likely caused the reduced RFC. In addition, the water flow transported some of the urchin feces from the start of the top tier to the end of the middle tier and into the bottom tier (J. Jacques, personal observations), meaning that waste from all preceding compartments passed through Positions 3 and 4 of the middle tier. This may have caused the lower feed consumption in these positions since reduced water quality from organic waste impacts urchin health and feed consumption (Mortensen et al. 2012).

The increase in RFC from the first to the fourth trial, followed by the plateau and subsequent decrease indicate that green sea urchin: (1) requires some acclimation to the formulated feed (Urchinomics V9.1.2); and (2) reaches a satiation point at ~4 wk beyond which the need to feed declines. Interestingly, although different feed versions and containment systems were used, this feeding trend is similar to that observed in Chapter II. Urchins in the turns of the raceway had a similar feeding trend, but a higher RFC, compared to urchins in the raceway straight segments. Variations in compartment shape and water flow may have caused this difference, with the turns having irregularly-shaped compartments and curved water flow (turning 360° over the three compartments) compared to the uniformly-shaped (rectangular) compartments and linear water flow in the raceway straight segments.

3.4.2 Aggregation

Urchins tend to aggregate together and limit movement in higher flow environments (Daggett et al. 2006, Devin 2002, Frey and Gagnon 2016); this was demonstrated in the present study as urchins aggregated to form few, large aggregations after initial individual placement, then

remained within these aggregations with limited movement throughout the duration of each trial. Urchins in the low and intermediate densities aggregated to form a higher number of smaller aggregations compared to urchins in the high density, reflecting the fact that urchins tend to more densely aggregate together with increasing density (Frey and Gagnon 2016). The lower number of aggregations in Positions 1 and 2 of both tiers compared to Positions 3 and 4 suggests that the higher flow in the upstream straight segment compared to the downstream straight segment in each tier (J. Jacques, personal observations) causes urchins to aggregate into fewer aggregations for more strength, supported by findings that urchins tend to form more dense aggregations at higher wave velocities (Frey and Gagnon 2016).

This higher flow in Positions 1 and 2 may have also caused the overall preference of the downstream area of the compartments compared to in Positions 3 and 4, with urchins avoiding the higher flow in the upstream area. Overall, the downstream area was preferred in the top tier whereas no preference was found in the middle tier, suggesting that the top tier has a higher overall water flow causing urchins to prefer the downstream area to avoid the higher flow. Similar percentages of urchins were found in each of the three compartment areas among all densities demonstrating that the differences in the number of urchins and in the space restriction within each compartment among densities did not impact the overall distribution of urchins within the compartments.

Overall, Positions 3 and 4 had a higher percentage of edge urchins compared to Positions 1 and 2 perhaps, again, due to the higher flow in Positions 1 and 2 since urchins have been found to avoid vertical surfaces at higher wave velocities due to lowered anchoring capacity (Frey and Gagnon 2016). This difference in positions was more apparent in the top tier perhaps due to the aforementioned higher overall flow compared to the middle tier. Similar percentages of urchins

were found on the edge of each compartment among all densities, despite the differences in the number of urchins and limited space along the edge in each compartment among densities. Although cultivated urchins tend to place themselves on the vertical sides of tanks (Daggett et al. 2006, Devin 2002), the maximum number of urchins that could fit on the edge (~100, 100, and 65%, for the low intermediate, and high urchin stocking densities, respectively) was not observed. This demonstrates that edge avoidance at higher wave velocities outweighed the tendency of urchins to position themselves on the edge, due to the overall high water flow throughout the raceway.

Despite the high density treatment having a higher number of urchins, more limited space, and a lower number of larger urchin aggregations compared to the intermediate and low densities, the percentage of urchins found in each of the three compartment areas and along compartment edges among densities were similar. This suggests that the strength of the flow is the main driver of these behaviours in the raceway rather than urchin density. The aggregation of urchins onto the edge and into dense aggregations led to empty space in the compartments, meaning that urchins can be stocked at an even higher density than was tested (up to ~150 urchins per compartment [~400 urchins m^{-2}]) while still maintaining a single layer of urchins. However, experiments would have to be performed to ensure that low mortality and high GSI is still maintained. Although elevated gonad growth and low mortality was maintained regardless of varying aggregation patterns in the present study, changing the containment system or increasing the urchin density may intensify these aggregation behaviours and cause feed access, gonad growth, or mortality issues. It is therefore important to consider these aggregation patterns along with their determinants (water flow, stocking density) in future urchin aquaculture studies.

3.4.3 Gonad yield

Gonadosomatic index (GSI) and change in GSI (ΔGSI) did not differ among urchin stocking densities nor among raceway locations despite differences in RFC and aggregation patterns, demonstrating that urchins can be stocked at the high density (10.5 kg urchins m^{-2}; 210 urchins m^{-2}) throughout the raceway for maximum production. An average GSI of ~21% was achieved after seven weeks of feeding, approximately double that of the GSI market target of ~10 to 15% (Pisces Consulting Limited 2014). Our results are comparable to a previous study in Norway on green sea urchins fed a prepared diet for eight weeks in a single-tiered raceway at 4.5 to 6.5°C from mid-November to early-January, resulting in a GSI of 17-18% with no differences among stocking densities (2.5, 3.7, and 7.3 kg urchins m^{-2}; Christiansen and Siikavuopio 2007). However, another study on green sea urchins fed a prepared diet for ~8.5 weeks in tanks at 8.0°C from early-August to early-October found that urchins had a higher GSI at the lowest stocking density of 3 kg urchins m^{-2} (~17%) compared to 8 kg urchins m^{-2} (Siikavuopio et al. 2007c). This demonstrates that our methodology in a tiered raceway system from late-January to mid-March was more efficient at maintaining gonad production at higher urchin stocking densities, since there was no difference in GSI between the low (2.5 kg urchins m^{-2}) and high (10.5 kg urchins m^{-2}) densities in the present study.

Discussion of the results from the bottom tier kelp-fed urchin will remain general since this portion of the experiment was largely exploratory; focus was mainly on the feed trials with a less rigorous level of attention dedicated towards the bottom tier. Urchins fed the kelp (*Laminaria digitata*) in the raceway achieved the GSI market target of ~10 to 15% (Pisces Consulting Limited 2014), reaching ~12%. To achieve a higher GSI, urchins should be fed more kelp than was provided (~70 kg) and/or fed over a longer period of time (greater than 6.5 weeks). The GSI of

~12% is comparable to the previous Newfoundland green sea urchin gonad enhancement study by Trueman (2019) during which a GSI of ~14% was achieved after 12 wk of feeding on *L. digitata* in rectangular tanks. The stocking density used in the present study (13 kg urchins m^{-2}; 260 urchins m^{-2}) was approximately 7.5 times higher than in the study by Trueman (2019), suggesting that the GSI we achieved was not negatively impacted by the elevated stocking density in the bottom tier. In fact, this tiered raceway system may be more beneficial as it took approximately half the time to achieve a similar GSI.

Urchins in the raceway straight segments and turns of the top two (feed) tiers had a similar GSI (and ΔGSI) indicating that the differences in RFC, compartment shapes, and water flow had no impact on resulting GSI. Urchins fed the formulated feed in the raceway had the highest GSI (and ΔGSI) of all diets and environments, demonstrating that the diet of feed in the raceway was significantly better at increasing urchin gonad mass compared to a diet of kelp in the field (kelp bed or urchin barrens) and in the bottom tier of the raceway. All urchin groupings achieved or surpassed the GSI market target of ~10 to 15% (Pisces Consulting Limited 2014) except for urchins from the barrens at the onset and end of the experiment, exhibiting low GSI likely as a result of food (e.g., kelp and other seaweed subsidy) shortage in the barrens from which they were taken (Blain and Gagnon 2014, Frey and Gagnon 2015). The negative ΔGSI (~ -38%) of urchins from the kelp bed is indicative of spawning in the field before the mid-March collection, which corresponds to the beginning of the natural peak spawning period (March to May) of the green sea urchin in Newfoundland (Himmelman 1978, Keats et al. 1984, Scheibling and Hatcher 2007). There is no indication of spawning in the barrens however, as GSI increased rather than decreased between collections.

3.4.4 Conclusion and future research directions

The present study demonstrates the efficiency of the tiered raceway system in producing marketable green sea urchin gonads in a short amount of time, especially when paired with a diet of formulated feed. Despite differences in urchin aggregation, feed consumption, and water flow, gonad yield throughout the raceway and in all three urchin stocking densities was consistent, demonstrating that urchins can be stocked at high densities throughout the raceway to maximize production. Future studies on this raceway system should measure GSI at multiple intervals throughout the experiment to determine the minimum amount of time required to reach market GSI, which can then be implemented to minimize production time. Future studies should also examine even higher urchin stocking densities, to determine the optimal density that maximizes gonad yield while still maintaining low mortality. Knowledge gained from these findings will contribute to the development of efficient tiered raceway systems for Newfoundland green sea urchin gonad enhancement.

CHAPTER IV

Summary

4.1 Overall objective of the study

Sea urchin gonads are increasing in popularity worldwide and can fetch a high price on seafood markets, however market demands are not met (Explorations Unlimited Inc. 2006, Stefánsson et al. 2017, Sun and Chiang 2015). The abundance of green sea urchin (*Strongylocentrotus droebachiensis*) along the coast of Newfoundland represents an untapped potential resource that can be used, through gonad enhancement programs, to help meet the global market demand and for communities to profit from this valuable resource. However, research is still needed on the ideal conditions for gonad enhancement of the Newfoundland green sea urchin since results may vary among geographical populations and the province is in need of science-based evidence that its green sea urchin resources are indeed amenable to gonad enhancement. The present study was the second of a planned series exploring the potential for a sustainable green sea urchin industry in eastern Newfoundland, following the study by Trueman (2019). Trueman (2019) examined green sea urchin gonad enhancement using natural kelp diets whereas the present study used formulated feeds.

The objective of this thesis was to study gonad enhancement of green sea urchin from southeastern Newfoundland to determine the conditions and systems that optimize gonad production when using formulated feeds. Specifically, (1) the effects of water temperature and proximity to spawning on green sea urchin feed consumption, feces production, physiological condition, gonad yield, and gonad taste were assessed using two 4- to 8-wk experiments on urchins collected before and during the spawning period, and fed a formulated feed at different water temperatures in conical tanks; and (2) the effects of stocking density on green sea urchin feed consumption, aggregation, and gonad yield in a tiered raceway were assessed using a 7-wk experiment on urchins fed a diet of formulated feed or a diet of kelp at different stocking densities

in a tiered raceway system. Knowledge of efficient water temperatures, seasonal timings, stocking densities, and containment system designs gained from the present study can be used by green sea urchin producers worldwide to implement successful formulated-feed-based gonad enhancement programs. Our success using land-based containment systems may also help to inspire and inform the future development of green sea urchin gonad enhancement industries in small, rural Newfoundland communities, which would create jobs and promote their economies.

4.2 Summary of the conical tank experiments

In Chapter II, we carried out two gonad enhancement experiments from mid-February to mid-June, with Newfoundland green sea urchins fed with a fish-meal-based feed (Nofima V9). Both experiments were carried out in commercially available, low-flow conical tanks and utilized urchins taken from barren grounds. The experiments differed in duration (4 or 8 wk) and timing of urchin collection from the field (just before [mid-February] or within [mid-April] the spawning period). In both experiments, urchins were exposed to one of three water temperatures (1, 3, or 6°C); feed consumption and feces production were respectively measured weekly and biweekly; and gonad yield and righting time were measured after 4 and 8 wk. Gonad taste was assessed after the 8-wk experiment. This design allowed us to test the suitability of the feed for gonad enhancement at varying temperatures, at a time of the year when natural gonad growth is nearly arrested, potentially limiting feed effects.

A strong relationship was found between relative feed consumption (RFC) and dry relative feces production (RFP); both RFC and RFP were highest at the maximum temperature of 6°C in the two experiments, and both were overall higher in urchins collected within the spawning period (ripe urchins) compared to urchins collected before the spawning period (unripe urchins).

Gonadosomatic index (GSI) increased with increasing temperature in both experiments, with unripe and ripe urchins achieving a GSI of ~10 and 12%, respectively, after 4 wk at 6°C, and ripe urchins achieving a higher GSI compared to unripe urchins. Urchins reached a maximum GSI of ~19% after 8 wk at 6°C. The majority (over 74%) of urchins righted (i.e. flipped from their aboral to oral side) within 300 s at any of the three water temperatures in both experiments, and righting time was lowest at 6°C, decreasing with increasing temperature. Gonads from urchins fed at 6°C were ranked as more bitter than gonads from field urchins, with about twice as many bitterness judgements compared to at 1 and 3°C.

4.3 Summary of the tiered raceway experiment

In Chapter III, we carried out a 7-wk experiment from late-January to mid-March with Newfoundland green sea urchins taken from barren grounds, and fed a fish-meal-based feed (Urchinomics V9.1.2) at three different stocking densities (2.5, 6.5, and 10.5 kg urchins m^{-2}) in a tiered raceway system. One ~72-hr trial was run each week, with feed consumption measured at the end of each trial, aggregation recorded throughout each trial, and gonadosomatic index (GSI) measured at the end of the 7-wk experiment. We also carried out concurrent trials with urchins fed kelp (*Laminaria digitata*) in the raceway at a stocking density of 13 kg urchins m^{-2}, for gonad yield comparisons. This design allowed us to test the suitability of the raceway for gonad enhancement, and to determine the optimal stocking density and location within the raceway that maximizes gonad yield.

Relative feed consumption (RFC) did not differ among the three urchin stocking densities, but was lowest in urchins located further downstream in the raceway. Urchins aggregated together on both the horizontal bottom and vertical edges of the raceway. Aggregation patterns varied

according to stocking density and position within the raceway, with urchins forming the densest aggregations at the highest stocking density (10.5 kg urchins m^{-2}), and in areas of the raceway with higher water flow. Urchins fed the formulated feed had a similar GSI among all three stocking densities and in all locations of the raceway, achieving ~21%, whereas urchins fed the kelp had a significantly lower GSI in comparison, achieving ~12%.

4.4 Importance of the study

Chapter II demonstrated that marketable green sea urchin gonads can be achieved in as little as four weeks at 6°C using a formulated feed (Nofima V9) in standard conical tanks, reaching the market gonadosomatic index (GSI) of ~10 to 15% (Pisces Consulting Limited 2014). Implementing similar conditions would allow urchin producers to dramatically accelerate production and reduce the associated costs of prolonged feeding and system maintenance. The present study supports previous findings that an increase in water temperature causes an increase in sea urchin feed consumption and gonad yield (Garrido and Barber 2001, Scheibling and Hatcher 2007, Siikavuopio et al. 2006, Siikavuopio et al. 2008); using land-based systems to control water temperature is therefore advantageous since elevated water temperatures will accelerate the gonad production process. The present study also provides evidence of successful gonad enhancement during the urchin's peak spawning period, at a time when natural gonad growth is nearly arrested, all while avoiding spawning in the tanks. This knowledge allows producers to continue production without having to halt during the spawning season.

Chapter III demonstrated that marketable green sea urchin gonads can be achieved in less than seven weeks at 6°C using a formulated feed (Urchinomics V9.1.2) in a tiered raceway system. After the 7-wk experiment, gonad yield was approximately double that of the market GSI, and was

similar among densities and throughout the raceway, regardless of differences found in feed consumption and aggregation patterns. These findings demonstrate that gonad enhancement is efficient and consistent in this tiered raceway system, and that the highest urchin density tested (10.5 kg urchins m^{-2}) can be used to maximize production, all while avoiding mortality which was negligible. Keeping in mind that the kelp portion of the present study was largely exploratory, results demonstrate that market GSI was achieved with the kelp diet in the raceway, but that the formulated feed diet was more successful, since urchins fed the feed reached approximately double the GSI in a similar amount of time. Nonetheless, these findings show that gonad enhancement in a tiered raceway system can be successful with varying urchin diets. Tiered raceways present several advantages over other tank designs, including better vertical space utilization and water use because the same water flows successively through multiple tiers (Daggett et al. 2006); these advantages in combination with the high and consistent GSI achieved in the present study support the use of tiered raceways for gonad enhancement programs.

The present study is the first to research gonad enhancement in eastern Newfoundland using formulated feeds, following previous successful research in the area using natural kelp diets by Trueman (2019). Trueman (2019) recently examined green sea urchin gonad enhancement in Newfoundland with three locally abundant kelp species as feed options, and found that *Laminaria digitata* enhanced gonads to within the GSI market target of ~10 to 15% (Pisces Consulting Limited 2014) in 12 wk. We achieved marketable urchin gonads in approximately half the time compared to Trueman (2019), demonstrating the time saved by using formulated feeds instead of kelp for gonad production. We achieved successful gonad enhancement in just a few weeks using formulated feeds in both the standard conical tanks and the tiered raceway system.

These findings contribute to knowledge and advancements needed to develop an efficient green sea urchin industry in Newfoundland.

4.5 Future directions

To better determine effects of water temperature on green sea urchin gonad enhancement, experiments similar to those in Chapter II should be performed at temperatures above 6°C. Results will help determine the temperature that maximizes gonad yield as well as the thermal tolerance limit at which gonad production begins to decrease and mortality increases to a detrimental level. Experiments in Chapter II demonstrated successful gonad enhancement during late winter to late spring, the most challenging time of year because of the risk of spawning and since natural gonad growth is nearly arrested. Future Newfoundland green sea urchin gonad enhancement experiments should therefore be run at other times throughout the year, to examine gonad production without this high risk and natural growth limitations. Green sea urchin feed consumption and gonad growth vary seasonally (Himmelman 1984, Scheibling and Hatcher 2007, Siikavuopio et al. 2007a), so these future experiments run at various times of the year will help determine the optimal timing for gonad enhancement.

In Chapter III, gonad yield was only measured at the end of the 7-wk experiment, at which point urchins exceeded the market GSI. Additional gonad enhancement studies in tiered raceway systems should measure green sea urchin gonad yield at various times throughout the experiment to examine gonad growth rate over time, and to determine at what point market GSI is achieved. The minimum time required to reach market GSI can then be implemented to reduce production time. Gonad enhancement studies should also be performed with raceway stocking densities above the maximum tested (10.5 kg urchins m^{-2}), to identify the highest density that maintains a high

urchin gonad production and yield, while still maintaining low mortality. More thorough research on the use of kelp diets in the tiered raceway should also be performed as the kelp portion of the present study was largely exploratory and requires further examination for more detailed results.

Methods to use sea urchin aquaculture production wastes (fecal matter, tests, etc.) such as simultaneously growing other organisms that feed on detritus, should also be developed to better manage waste production and improve the sustainability of gonad enhancement practices. Although both fish-based formulated feed versions used in the present study (Nofima V9 and Urchinomics V9.1.2) allowed urchins to achieve marketable gonad yields in just a few weeks time, newer feed versions that contain more sustainable ingredients should be used in future studies that improve the quality of the resulting gonads, to fix the bitterness issue discussed in Chapter II. A companion study examining drivers of gonad taste in Newfoundland green sea urchins fed with the Nofima V9 feed or either of two kelp-based variants of the feed, highlights the benefits of switching from an animal-protein-based to a kelp-protein-based formulation (Pellerin 2020). Additionally, kelp-based feeds are more sustainable, and therefore should be used in future studies to promote the sustainability of the green sea urchin gonad enhancement industry.

LITERATURE CITED

Andrew NL, Agatsuma Y, Ballesteros E, Bazhin AG, Creaser EP, Barnes DKA, Botsford LW, Bradbury A, Campbell A, Dixon JD, Einarsson S, Gerring PK, Hebert K, Hunter M, Hur SB, Johnson CR, Juinio-Menez MA, Kalvass P, Miller RJ, Moreno CA, Palleiro JS, Rivas D, Robinson SML, Schroeter SC, Steneck RS, Vadas RL, Woodby DA & Xiaoqi Z. 2002. Status and management of world sea urchin fisheries. Oceanography and Marine Biology 40:343-425.

Azad AK, Pearce CM, McKinley RS. 2011. Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). Aquaculture 317:187-196.

Blain C, Gagnon P. 2013. Interactions between thermal and wave environments mediate intracellular acidity (H_2SO_4), growth, and mortality in the annual brown seaweed *Desmarestia viridis*. Journal of Experimental Marine Biology and Ecology 440:176-184.

Blain C, Gagnon P. 2014. Canopy-forming seaweeds in urchin-dominated systems in eastern Canada: structuring forces or simple prey for keystone grazers? Plos One 9:e98204.

Boyd CE, McNevin AA, Tucker CS. 2019. Resource use and the environment. In: Aquaculture: farming aquatic animals and plants. JS Lucas, PC Southgate, CS Tucker, eds. West Sussex: John Wiley & Sons Ltd. pp. 93-112.

Brant R. 1990. Assessing proportionality in the proportional odds model for ordinal logistic regression. Biometrics 46:1171-1178.

Brown JH, Gillooly JF, Allen AP, Savage VM. 2004. Towards a metabolic theory of ecology. Ecology 85:1771-1789.

Caines S, Gagnon P. 2012. Population dynamics of the invasive bryozoan *Membranipora membranacea* along a 450-km latitudinal range in the subarctic northwestern Atlantic. Marine Biology 159:1817-1832.

Carey N, Harianto J, Byrne M. 2016. Sea urchins in a high-CO_2 world: partitioned effects of body size, ocean warming and acidification on metabolic rate. Journal of Experimental Biology 219:1178-1186.

Carrier TJ, Eddy SD, Redmond S. 2017. Solar-dried kelp as potential feed in sea urchin aquaculture. Aquaculture International 25:355-366.

Christiansen JS, Siikavuopio SI. 2007. The relationship between feed intake and gonad growth of single and stocked green sea urchin (*Strongylocentrotus droebachiensis*) in a raceway culture. Aquaculture 262:163-167.

Cuthbert FM, Hooper RG, McKeever T. 1995. Sea urchin feeding and ranching experiments. St. John's, NL: Canadian Centre for Fisheries Innovation.

Daggett TL, Pearce CM, Robinson SMC. 2006. A comparison of three land-based containment systems for use in culturing green sea urchins, *Strongylocentrotus droebachiensis* (Muller) (Echinodermata : Echinoidea). Aquaculture Research 37:339-350.

Devin MG. 2002. Land-based echinoculture: a novel system to culture adult sea urchins. In: The sea urchin: from basic biology to aquaculture. Y. Yokota, V Matranga, Z Smolenicka, eds. Lisse: A.A. Balkema Publishers. pp. 145-159.

DFO. 2018. Seafisheries landed quantity by province, 2018. Ottawa: Department of Fisheries and Oceans.

Explorations Unlimited Inc. 2006. Sea urchin fishery profiles. British Columbia: Pacific Urchin Harvesters Association (PUHA) and West Coast Green Urchin Association (WCGUA).

FAO. 2016. Food and Agriculture Organization fisheries statistics. Rome: Food and Agriculture Organization of the United Nations.

FAO. 2018. The state of world fisheries and aquaculture: meeting the sustainable development goals. Rome: Food and Agriculture Organization of the United Nations.

FAO. 2020. The state of world fisheries and aquaculture: sustainability in action. Rome: Food and Agriculture Organization of the United Nations.

Frey DL, Gagnon P. 2015. Thermal and hydrodynamic environments mediate individual and aggregative feeding of a functionally important omnivore in reef communities. Plos One 10:e0118583.

Frey DL, Gagnon P. 2016. Spatial dynamics of the green sea urchin *Strongylocentrotus droebachiensis* in food-depleted habitats. Marine Ecology Progress Series 552:223-240.

Gagnon P, Himmelman JH, Johnson LE. 2004. Temporal variation in community interfaces: kelp bed boundary dynamics adjacent to persistent urchin barrens. Marine Biology 144:1191-1203.

Garrido CL, Barber BJ. 2001. Effects of temperature and food ration on gonad growth and oogenesis of the green sea urchin, *Strongylocentrotus droebachiensis*. Marine Biology 138:447-456.

Grosjean P, Spirlet C, Gosselin P, Vaitilingon D, Jangoux M. 1998. Land-based, closed-cycle echinoculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): a long-term experiment at a pilot scale. Journal of Shellfish Research 17:1523-1604.

Han G, Loder JW, Smith PC. 1999. Seasonal-mean hydrography and circulation in the Gulf of St. Lawrence and on the eastern Scotian and southern Newfoundland shelves. Journal of Physical Oceanography 29:1279-1301.

Himmelman JH. 1978. Reproductive cycle of the green sea urchin, *Strongylocentrotus droebachiensis*. Canadian Journal of Zoology 56:1828-1836.

Himmelman JH. 1984. Urchin feeding and macroalgal distribution in Newfoundland, eastern Canada. Naturaliste Canadien 111:337-348.

Himmelman JH, Steele DH. 1971. Foods and predators of the green sea urchin *Strongylocentrotus droebachiensis* in Newfoundland waters. Marine Biology 9:315-322.

James P, Evensen T, Samuelsen A. 2017. Commercial scale sea urchin roe enhancement in Norway: enhancement, transport and market assessment. Tromsø: Nofima.

James P, Noble C, Hannon C, Stefánsson G, Þórarinsdóttir G, Ziemer N, Lochead J. 2016. Sea urchin fisheries, management and policy review. Tromsø: Nofima.

James P, Siikavuopio SI. 2012. The effect of continuous and intermittent feeding regimes on survival and somatic and gonadal growths of the sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 364:173-179.

James P, Siikavuopio SI. 2015. The effects of tank system, water velocity and water movement on survival, somatic and gonad growth of juvenile and adult green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture Research 46:1501-1509.

Jobling M. 1994. Biotic factors and growth performance. In: Fish bioenergetics. Fish and fisheries series, vol. 13. M Jobling, ed. London: Chapman and Hall. pp. 155-201.

Keats DW, Steele DH, South GR. 1984. Depth-dependent reproductive output of the green sea urchin, *Strongylocentrotus droebachiensis* (O. F. Müller), in relation to the nature and availability of food. Journal of Experimental Marine Biology and Ecology 80:77-91.

Keesing JK, Hall KC. 1998. Review of harvests and status of world sea urchin fisheries points to opportunities for aquaculture. Oceanography and Marine Biology 13:213-286.

Kleitman N. 1941. The effect of temperature on the righting of echinoderms. Biological Bulletin 80:292-298.

Lawless HT, Heymann H. 2010. Sensory evaluation of food: principles and practices. New York: Springer Science & Business Media.

Lawrence JM. 1975. On the relationships between marine plants and sea urchins. Oceanography and Marine Biology 13:213-286.

Le Gall P. 1990. Culture of echinoderms. In: Aquaculture, vol. 1. G Barnabé, ed. New York: Ellis Horwood. pp. 443-462.

McCullagh P. 1980. Regression models for ordinal data. Journal of the Royal Statistical Society Series B Methodological 42:109-142.

Mortensen A, Siikavuopio SI, James P. 2012. Water quality requirements for culture of the green sea urchin, *Strongylocentrotus droebachiensis*. World Aquaculture 43:46-50.

Murray HM, Ollerhead LMN. 2018. A comparison of ArcGIS and Image J software tools for calculation of gonad volume fraction (GVF) from histological sections of blue mussel cultured in deep and shallow sites on the northeast coast of Newfoundland. Canadian Technical Report of Fisheries and Aquatic Sciences 3275:vi-17.

Pearce CM, Daggett TL, Robinson SMC. 2002. Effect of protein source ratio and protein concentration in prepared diets on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis.* Aquaculture 214:307-332.

Pearce CM, Daggett TL, Robinson SMC. 2003. Effects of starch type, macroalgal meal source, and β-carotene on gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis* (Müller), fed prepared diets. Journal of Shellfish Research 22:505-519.

Pearce CM, Daggett TL, Robinson SMC. 2004. Effect of urchin size and diet on gonad yield and quality in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 233:337-367.

Pearce CM, Robinson SMC. 2010. Recent advances in sea-urchin aquaculture and enhancement in Canada. Bulletin of the Aquaculture Association of Canada 108:38-48.

Pellerin É. 2020. Drivers of gonad taste in green sea urchin (*Strongylocentrotus droebachiensis*): a comparison of amino acid profiles among urchins fed with advanced formulated feeds. B.Sc. Hons Thesis. Memorial University of Newfoundland. St. John's. pp. 61.

Pisces Consulting Limited. 2014. Sea urchin fishery review. Paradise, NL: Department of Fisheries and Aquaculture.

Prato E, Fanelli G, Angioni A, Biandolino F, Parlapiano I, Papa L, Denti G, Secci M, Chiantore M, Kelly MS, Ferranti MP, Addis P. 2018. Influence of a prepared diet and a macroalga (*Ulva* sp.) on the growth, nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus.* Aquaculture 493:240-250.

Quinn GP, Keough MJ. 2002. Experimental design and data analysis for biologists. New York: Cambridge University Press.

R Core Team. 2018. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Robinson SMC. 2004. The evolving role of aquaculture in the global production of sea urchin. In: Sea urchins-fisheries and ecology: proceedings of the international conferences on sea urchin fisheries and aquaculture. JM Lawrence, O Guzmán, eds. Lancaster: DEStech Publications Inc. pp. 343-357.

Robinson SMC, Castell JD, Kennedy EJ. 2002. Developing suitable colour in the gonads of cultured green sea urchins (*Strongylocentrotus droebachiensis*). Aquaculture 206:289-303.

Sauchyn LK, Lauzon-Guay J-S, Scheibling RE. 2011. Sea urchin fecal production and accumulation in a rocky subtidal ecosystem. Aquatic Biology 13:215-223.

Sauchyn LK, Scheibling RE. 2009. Fecal production by sea urchins in native and invaded algal beds. Marine Ecology Progress Series 396:35-48.

Scheibling RE, Hatcher BG. 2007. Ecology of *Strongylocentrotus droebachiensis*. In: Edible sea urchins: biology and ecology. JM Lawrence, ed. Amsterdam: Elsevier. pp. 353-392.

Scheibling RE, Hennigar AW, Balch T. 1999. Destructive grazing, epiphytism, and disease: the dynamics of sea urchin-kelp interactions in Nova Scotia. Canadian Journal of Fisheries and Aquatic Science 56:2300-2314.

Shpigel M, McBride SC, Marciano S, Ron S, Ben-Amotz A. 2005. Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. Aquaculture 245:101-109.

Siikavuopio SI, Christiansen JS, Dale T. 2006. Effects of temperature and season on gonad growth and feed intake in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 255:389-394.

Siikavuopio SI, Christiansen JS, Sæther B-S, Dale T. 2007a. Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 272:328-334.

Siikavuopio SI, Dale T, Carlehög M. 2007b. Sensory quality of gonads from the green sea urchin, *Strongylocentrotus droebachiensis*, fed different diets. Journal of Shellfish Research 26:637-643.

Siikavuopio SI, Dale T, Foss A, Mortensen A. 2004. Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin *Strongylocentrotus droebachiensis*. Aquaculture 242:313-320.

Siikavuopio SI, Dale T, Mortensen A. 2007c. The effects of stocking density on gonad growth, survival and feed intake of adult green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 262:78-85.

Siikavuopio SI, Dale T, Mortensen A, Foss A. 2007d. Effects of hypoxia on feed intake and gonad growth in the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 266:112-116.

Siikavuopio SI, Mortensen A, Christiansen JS. 2008. Effects of body weight and temperature on feed intake, gonad growth and oxygen consumption in green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 281:77-82.

Siikavuopio SI, Mortensen A, Dale T, Foss A. 2007e. Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 266:97-101.

Snedecor G, Cochran W. 1994. Statistical methods, 8th edition. DH Jones, ed. Ames: Iowa State University Press.

Sokal RR, Rohlf FJ. 2012. Biometry: the principles and practice of statistics in biological research. New York: W.H. Freeman and Co.

Stefánsson G, Kristinsson H, Ziemer N, Hannon C, James P. 2017. Markets for sea urchins: a review of global supply and markets. Reykjavík: Matís.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ. 2002. Kelp forest ecosystems: biodiversity, stability, resilience and future. Environmental Conservation 29:436-459.

Sun J, Chiang F. 2015. Use and expoloitation of sea urchins. In: Echinoderm aquaculture. N Brown, S Eddy, eds. Hoboken: John Wiley & Sons, Inc. pp. 25-44.

Suskiewicz TS, Johnson LE. 2017. Consumption rates of a key marine herbivore: a review of the extrinsic and intrinsic control of feeding in the green sea urchin. Marine Biology 164:1-13.

Trueman SC. 2019. A pilot study on gonad enhancement in kelp-fed Newfoundland green sea urchin, *Strongylocentrotus droebachiensis*. M.Sc. Thesis. Memorial University of Newfoundland. St. John's. pp. 98.

Unuma T. 2002. Gonadal growth and its relationship to aqua-culture in sea urchins. In: The sea urchin: from basic biology to aquaculture. Y Yukio, V Matranga, Z Smolenicka, eds. The Netherlands: A.A. Balkema Publishers. pp. 115-128.

Wei J, Zhang L, Zhao C, Feng W, Sun P, Chang Y. 2016. Correlation analyses of covering and righting behaviors to fitness related traits of the sea urchin *Glyptocidaris crenularis* in different environmental conditions. Chinese Journal of Oceanology and Limnology 34:1183-1190.

Whitaker R, Quinlan W, Daley C, Parsons J. 1997. Developing markets for feed lot sea urchins. Bulletin of the Aquaculture Association of Canada 97:42-44.

Yamaguchi S. 1991. Basic properties of umami and effects on humans. Physiology & Behavior 49:833-841.

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM. 2009. Mixed Effects Models and Extensions in Ecology with R. New York: Springer Science+Business Media, LLC.

APPENDIX A

Water temperature in the experimental tanks during the acclimation and experimental phases of Experiment 1 and Experiment 2

To characterize the thermal environment to which urchins were exposed, we recorded water temperature in the experimental tanks during the acclimation and experimental phases of Experiment 1 (4-wk long, carried out with unripe urchins) and Experiment 2 (8-wk long, carried out with ripe urchins). The experimental tanks in both experiments were supplied with flow-through seawater (~3 L min^{-1}) at three different temperatures: ambient (~1), 3, and 6°C; chosen to represent sea surface temperature for the region during winter (1°C), early spring (3°C), and early summer (6°C; Blain and Gagnon 2013, Frey and Gagnon 2016). Both experiments were preceded by a 7-d acclimation phase during which urchins in the experimental tanks underwent gradual water heating and/or cooling cycles (see section 2.2.2 for details of cycles). Water temperature was recorded every 10 min with one temperature and light logger (HOBO Pendant; Onset Computer Corporation) placed on the bottom of one randomly chosen tank from each temperature treatment.

Water temperature during the acclimation phase increased similarly steadily within and between each temperature treatment of both experiments (Figure A.1). Temperature in each treatment was quite stable throughout the experimental phases of both experiments, with a slight, inevitable increase over time in the ambient temperature treatment in Experiment 2 (Figure A.1). Mean water temperature in the 1 (ambient), 3, and 6°C temperature treatments was respectively 0.7, 3.2, and 6.4°C for Experiment 1, and 1.3, 3.1, and 6.3°C for Experiment 2 (Table A.1).

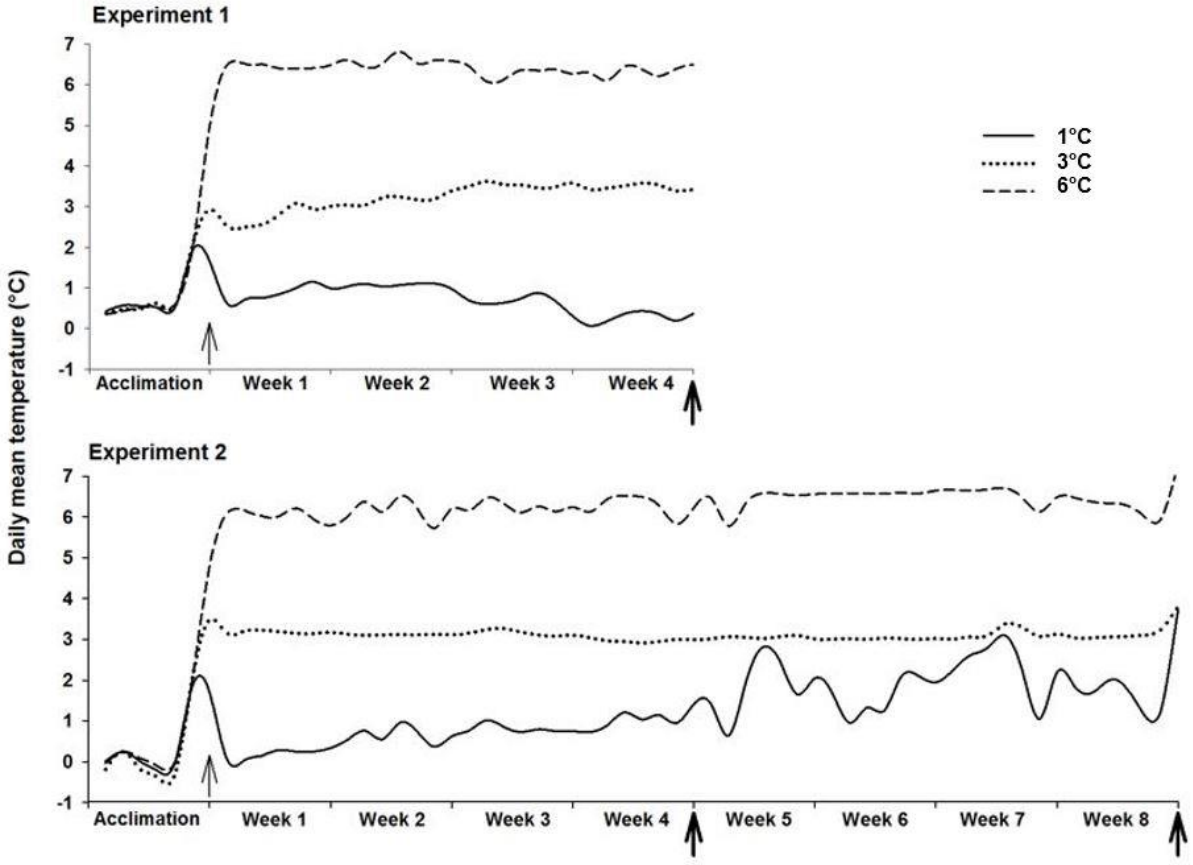

Figure A.1 Daily mean water temperature for each nominal temperature treatment (1, 3, and 6°C) throughout the 7-d acclimation and 4- (Experiment 1) or 8- (Experiment 2) wk experimental phases. Thin arrows above the x-axis in both experiments mark the end of the acclimation phase and onset of the experimental phase (see section 2.2.2 for methodological details). Thick arrows below the x-axes indicate timing of urchin collections (for comparison purposes) at the end of the 4th week in both experiments and end of 8th week in Experiment 2.

Table A.1 Mean daily mean water temperature for each nominal temperature treatment (1, 3, and 6°C) throughout the acclimation and experimental phases of Experiment 1 (4-wk long, carried out with unripe urchins) and Experiment 2 (8-wk, carried out with ripe urchins), broken down by relevant procedural time blocks (see section 2.2.2 for methodological details).

	Experiment	Treatment	Mean (±SE) water temperature (°C)
Acclimation phase	1 (days 1-5)	1°C	0.5 (0.0)
		3°C	0.5 (0.0)
		6°C	0.5 (0.0)
	1 (day 6)	1°C	1.9 (0.1)
		3°C	2.0 (0.1)
		6°C	2.0 (0.1)
	1 (day 7)	1°C	1.6 (0.1)
		3°C	2.9 (0.0)
		6°C	5.0 (0.1)
	2 (days 1-5)	1°C	0.0 (0.0)
		3°C	-0.2 (0.0)
		6°C	0.0 (0.0)
	2 (day 6)	1°C	1.8 (0.1)
		3°C	2.0 (0.2)
		6°C	2.0 (0.1)
	2 (day 7)	1°C	1.7 (0.1)
		3°C	3.5 (0.0)
		6°C	4.8 (0.1)
Experimental phase	1 (weeks 1-4)	1°C	0.7 (0.0)
		3°C	3.2 (0.0)
		6°C	6.4 (0.0)
	2 (weeks 1-4)	1°C	0.7 (0.0)
		3°C	3.1 (0.0)
		6°C	6.2 (0.0)
	2 (weeks 5-8)	1°C	1.9 (0.0)
		3°C	3.1 (0.0)
		6°C	6.5 (0.0)

Table A.1 ***Continued***

Experiment	Treatment	Mean (±SE) water temperature (°C)
2 (weeks 1-8)	1°C	1.3 (0.0)
	3°C	3.1 (0.0)
	6°C	6.3 (0.0)

APPENDIX B

Summary of sea urchin characteristics, feed consumption, and feces production in Experiment 1 and Experiment 2

Table B.1 Mean (±SE) test diameter, body wet weight, gonad wet weight, gonadosomatic index (GSI), change in GSI (ΔGSI), and righting time of green sea urchins sampled from the field (N=50) and from each water temperature treatment (N=50 per treatment) for Experiment 1 (onset and at 4 wk [end]) and Experiment 2 (onset and at 4 and 8 wk [end]).

Exp	Sampling time	Treatment	Test diameter (mm)	Body wet weight (g)	Gonad wet weight (g)	GSI (%)	ΔGSI (%)	Righting time (s)
1	Onset	Field	48.1 (0.3)	48.7 (1.1)	1.9 (0.2)	4.0 (0.3)	-	-
	4 wk	Field	50.1 (0.3)	55.3 (1.0)	2.4 (0.2)	4.5 (0.4)	11.7 (8.8)	230.4 (10.5)
		1°C	48.3 (0.3)	49.3 (1.0)	2.8 (0.2)	5.7 (0.4)	43.9 (10.0)	199.7 (8.9)
		3°C	48.5 (0.4)	50.1 (1.3)	3.3 (0.2)	6.7 (0.3)	68.8 (8.0)	153.5 (7.9)
		6°C	49.4 (0.4)	53.5 (1.3)	5.1 (0.3)	9.6 (0.5)	141.4 (13.2)	132.8 (10.1)
2	Onset	Field	49.6 (0.4)	53.8 (1.3)	4.2 (0.3)	7.9 (0.7)	-	-
	4 wk	Field	49.1 (0.8)	50.7 (1.1)	2.0 (0.2)	4.0 (0.3)	-50.0 (4.1)	164.6 (8.8)
		1°C	48.9 (0.3)	51.7 (1.0)	4.9 (0.2)	9.4 (0.4)	18.6 (5.2)	181.2 (8.3)
		3°C	49.6 (0.4)	53.3 (1.0)	5.2 (0.3)	9.9 (0.5)	24.9 (6.0)	162.2 (6.3)
		6°C	49.8 (0.3)	56.0 (1.1)	6.6 (0.4)	11.9 (0.7)	50.2 (9.1)	138.5 (7.7)
2	8 wk	Field	49.3 (0.4)	52.8 (1.2)	2.7 (0.2)	5.0 (0.4)	-37.0 (4.8)	158.7 (9.3)
		1°C	48.7 (0.3)	52.1 (0.9)	6.7 (0.3)	12.8 (0.6)	61.8 (7.5)	146.8 (7.5)
		3°C	49.4 (0.4)	54.7 (1.2)	7.4 (0.4)	13.4 (0.7)	68.8 (8.5)	127.0 (7.5)
		6°C	50.2 (0.4)	57.8 (1.3)	11.1 (0.5)	19.2 (0.7)	142.3 (8.8)	85.8 (4.1)

Table B.2 Mean (±SE) relative feed consumption (RFC, in g feed kg^{-1} urchin) and relative feces production (RFP; wet and dry weights, in g feces kg^{-1} urchin) for each water temperature treatment (N=10 per sampling week) in Experiment 1.

Parameter	Treatment	Week 1	Week 2	Week 3	Week 4	Mean
RFC	1°C	21.0 (0.4)	22.6 (0.7)	21.5 (1.2)	15.1 (0.8)	20.0 (0.6)
	3°C	25.9 (0.5)	27.7 (1.3)	22.6 (2.1)	28.7 (1.6)	26.2 (0.8)
	6°C	33.0 (0.8)	34.6 (0.7)	42.5 (1.2)	51.0 (0.6)	40.3 (1.2)
RFP (wet)	1°C	17.4 (1.3)	13.4 (1.6)	12.3 (2.5)	7.0 (1.5)	12.5 (1.1)
	3°C	29.5 (2.1)	20.3 (3.6)	14.0 (1.9)	12.3 (1.7)	19.0 (1.6)
	6°C	31.5 (2.5)	17.9 (1.8)	28.6 (3.7)	30.4 (4.6)	27.1 (1.8)
RFP (dry)	1°C	2.1 (0.2)	1.7 (0.3)	1.7 (0.4)	1.0 (0.2)	1.6 (0.1)
	3°C	3.2 (0.2)	2.8 (0.5)	2.0 (0.3)	1.8 (0.2)	2.4 (0.2)
	6°C	3.6 (0.3)	2.5 (0.3)	4.0 (0.5)	4.2 (0.7)	3.6 (0.3)

Table B.3 Mean (±SE) relative feed consumption (RFC, in g feed kg^{-1} urchin) and relative feces production (RFP; wet and dry weights, in g feces kg^{-1} urchin) for each water temperature treatment (N=10 per sampling week) in Experiment 2.

Parameter	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Mean
RFC	1°C	23.0 (0.6)	19.3 (0.7)	22.9 (0.6)	22.4 (1.3)	37.3 (1.2)	32.9 (1.1)	32.7 (2.1)	30.4 (1.5)	27.6 (0.8)
	3°C	29.4 (0.9)	24.3 (1.6)	29.1 (1.4)	32.8 (1.5)	40.8 (1.7)	38.1 (1.6)	38.2 (1.7)	36.6 (1.9)	33.6 (0.8)
	6°C	40.5 (0.3)	47.1 (1.9)	51.4 (1.9)	61.1 (2.0)	76.3 (0.5)	73.8 (1.4)	68.4 (1.3)	57.9 (2.2)	59.6 (1.5)
RFP (wet)	1°C	13.6 (1.1)	17.1 (1.9)	16.7 (1.1)	30.4 (3.0)	48.0 (3.8)	33.1 (1.6)	40.0 (3.7)	54.2 (3.8)	31.6 (1.8)
	3°C	34.3 (2.8)	24.5 (3.0)	31.3 (3.8)	56.4 (2.7)	53.0 (2.9)	56.3 (3.4)	52.6 (4.0)	64.9 (3.2)	46.7 (1.9)
	6°C	51.0 (1.9)	61.2 (3.4)	85.0 (3.3)	113.0 (3.2)	125.0 (5.7)	127.1 (7.2)	112.8 (6.6)	101.9 (9.5)	97.1 (3.6)
RFP (dry)	1°C	1.8 (0.1)	2.3 (0.2)	2.5 (0.2)	4.3 (0.5)	7.2 (0.5)	5.3 (0.3)	5.8 (0.6)	7.6 (0.5)	4.6 (0.3)
	3°C	4.3 (0.3)	3.7 (0.4)	4.6 (0.5)	7.9 (0.4)	7.9 (0.4)	7.4 (0.5)	7.4 (0.6)	9.0 (0.5)	6.5 (0.3)
	6°C	6.9 (0.2)	9.0 (0.5)	11.5 (0.4)	15.5 (0.6)	17.2 (0.6)	16.9 (1.0)	15.5 (0.8)	14.0 (1.3)	13.3 (0.5)

APPENDIX C

Strength of relationships between sea urchin relative feces production (RFP) and relative feed consumption (RFC) during Experiment 1 and 2

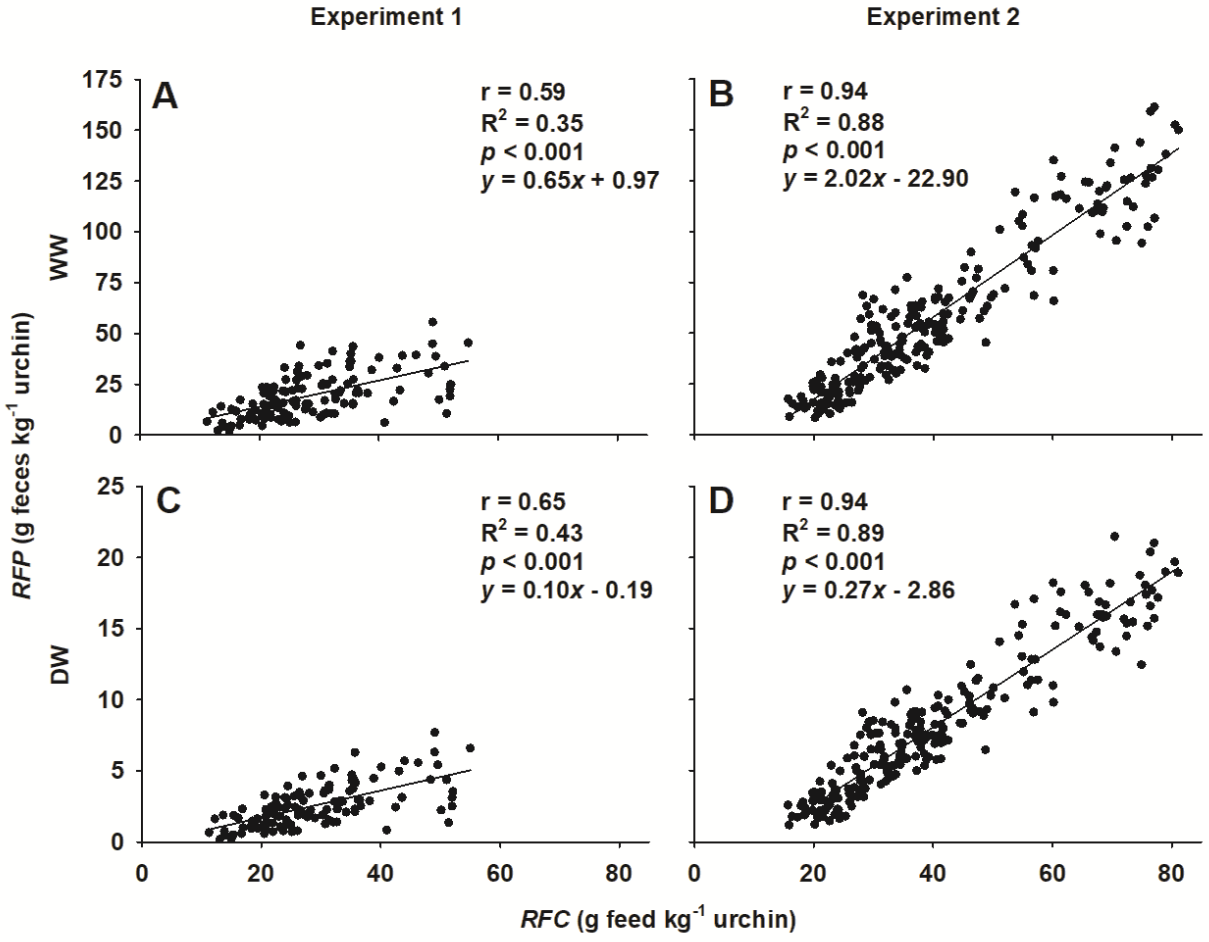

Figure C.1 Relationship (simple linear regression analysis) between green sea urchin's relative feces production (RFP) and relative feed consumption (RFC) during Experiment 1 (A, C; N=120 for each relationship) and Experiment 2 (B, D; N=240 for each relationship) based on feces wet (WW; A, B) and dry (DW; C, D) weights.

APPENDIX D

Summary of full model ANOVAs and multiple ordinal logistic regression analysis

Table D.1 Summary of two-way ANOVA (applied to raw data) examining the effect of Experiment (E; Experiment 1 and Experiment 2) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) at the end of the 4th experimental week (final week for Experiment 1 and midpoint week for Experiment 2) (see details of statistical analysis in section 2.2.8.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
E	1	93.0	38.5	<0.001
T	3	146.8	60.8	<0.001
E x T	3	17.2	7.1	<0.001
Error	72	2.4		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.2 Summary of two-way ANOVA (applied to raw data) examining the effect of Sampling time (S; 4th and 8th experimental weeks) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) during Experiment 2 (see details of statistical analysis in section 2.2.8.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
S	1	290.9	65.9	<0.001
T	3	423.2	95.9	<0.001
S x T	3	33.7	7.6	<0.001
Error	72	4.4		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.3 Summary of two-way ANOVA (applied to raw data) examining the effect of Experiment (E; Experiment 1 and Experiment 2) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the change in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) at the end of the 4th experimental week (final week for Experiment 1 and midpoint week for Experiment 2) (see details of statistical analysis in section 2.2.8.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
E	1	61712	78.9	<0.001
T	3	44831	57.3	<0.001
E x T	3	3927	5.0	<0.01
Error	72	782		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.4 Summary of two-way ANOVA (applied to raw data) examining the effect of Sampling time (S; 4th and 8th experimental weeks) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the change in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) during Experiment 2 (see details of statistical analysis in section 2.2.8.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
S	1	46180	65.9	<0.001
T	3	67180	95.9	<0.001
S x T	3	5350	7.6	<0.001
Error	72	700		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.5 Summary of two-way ANOVA (applied to raw data) examining the effect of Experiment (E; Experiment 1 and Experiment 2) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the proportion of green sea urchins (*Strongylocentrotus droebachiensis*) that successfully righted at the end of the 4th experimental week (final week for Experiment 1 and midpoint week for Experiment 2) (see details of statistical analysis in section 2.2.8.4).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
E	1	0.88	34.1	<0.001
T	3	0.54	20.8	<0.001
E x T	3	0.22	8.5	<0.001
Error	72	0.03		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.6 Summary of two-way ANOVA (applied to raw data) examining the effect of Sampling time (S; 4th and 8th experimental weeks) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the proportion of green sea urchins (*Strongylocentrotus droebachiensis*) that successfully righted during Experiment 2 (see details of statistical analysis in section 2.2.8.4).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
S	1	0.005	0.3	0.58
T	3	0.167	11.6	<0.001
S x T	3	0.006	0.4	0.75
Error	72	0.014		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.7 Summary of two-way ANOVA (applied to raw data) examining the effect of Experiment (E; Experiment 1 and Experiment 2) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the righting time of green sea urchins (*Strongylocentrotus droebachiensis*) that successfully righted at the end of the 4th experimental week (final week for Experiment 1 and midpoint week for Experiment 2) (see details of statistical analysis in section 2.2.8.4).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
E	1	5089.2	6.2	0.016
T	3	16151.5	19.5	<0.001
E x T	3	5356.6	6.5	<0.001
Error	70	827.3		
Corrected total	77			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.8 Summary of two-way ANOVA (applied to raw data) examining the effect of Sampling time (S; 4th and 8th experimental weeks) and Treatment (T; the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on the righting time of green sea urchins (*Strongylocentrotus droebachiensis*) that successfully righted during Experiment 2 (see details of statistical analysis in section 2.2.8.4).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
S	1	21793.8	41.7	<0.001
T	3	11282.9	21.6	<0.001
S x T	3	1578.5	3.0	0.035
Error	72	522.8		
Corrected total	79			

DF = degrees of freedom; MS = mean squares; *p* = p-value

Table D.9 Summary of multiple ordinal logistic regression (applied to raw data) examining the effect of Treatment (the three water temperature treatments; ~1, 3, and 6°C, and urchins from the field) on green sea urchin (*Strongylocentrotus droebachiensis*) gonad overall preference and taste at the end of Experiment 2 (see details of statistical analysis in section 2.2.8.5).

Parameter	**Source of variation**	**DF**	**LR χ^2**	***p***
Overall preference	Treatment	3	7.30	0.063
Taste	Treatment	3	9.33	0.025

DF = degrees of freedom; LR χ^2 = likelihood ratio chi-square; *p* = p-value

APPENDIX E

Water parameters in each tier and section of the raceway throughout the experiment

Table E.1 Mean (±SE) water temperature (°C) during the last six days of the 10-d acclimation phase), the 7-wk experimental phase, and the 6-d sampling phase of the feed trials measured every five minutes by two temperature loggers (N=576 each day per tier) placed in the center compartment of the turn in each tier (top, middle, and bottom).

Phase		Top	Middle	Bottom
Acclimation		6.2 (0.0)	6.3 (0.0)	6.3 (0.0)
Experimental week	1	6.2 (0.0)	6.3 (0.0)	6.4 (0.0)
	2	6.1 (0.0)	6.3 (0.0)	6.3 (0.0)
	3	6.0 (0.0)	6.2 (0.0)	6.2 (0.0)
	4	6.2 (0.0)	6.3 (0.0)	6.3 (0.0)
	5	6.1 (0.0)	6.3 (0.0)	6.3 (0.0)
	6	6.1 (0.0)	6.2 (0.0)	6.3 (0.0)
	7	6.0 (0.0)	6.2 (0.0)	6.2 (0.0)
	Total	6.1 (0.0)	6.3 (0.0)	6.3 (0.0)
Sampling		5.9 (0.01)	6.0 (0.0)	6.1 (0.0)

Table E.2 Sample size (N) and mean (±SE) water temperature (Temp), dissolved oxygen (DO), salinity (Sal), and pH for each position within the top and middle tiers (1-4 and the turn) and the bottom tier (compartments 1-3, before 1, and after 3) over the seven feed trials (see Figures 3.2 and 3.3 for spatial layouts of the raceway), measured with a YSI Professional Plus (Model Pro 10102030). See sections 3.2.4 and 3.2.5 for timing of readings.

Tier	N	Pos	Temp (ºC)	DO (%)	Sal (ppt)	pH
Top	70	1	5.9 (0.0)	125.3 (0.7)	31.4 (0.0)	8.3 (0.0)
Top	70	2	5.9 (0.0)	123.1 (0.7)	31.4 (0.0)	8.3 (0.0)
Top	70	Turn	6.0 (0.0)	121.6 (0.7)	31.4 (0.0)	8.3 (0.0)
Top	70	3	6.0 (0.0)	120.2 (0.6)	31.4 (0.0)	8.3 (0.0)
Top	70	4	6.0 (0.0)	118.3 (0.7)	31.4 (0.0)	8.3 (0.0)
Mean	350	-	6.0 (0.0)	121.7 (0.7)	31.4 (0.0)	8.3 (0.0)
Mid	70	1	6.1 (0.0)	107.1 (0.4)	31.4 (0.0)	8.3 (0.0)
Mid	70	2	6.1 (0.0)	106.0 (0.4)	31.4 (0.0)	8.3 (0.0)
Mid	70	Turn	6.1 (0.0)	105.3 (0.4)	31.4 (0.0)	8.3 (0.0)
Mid	70	3	6.1 (0.0)	104.3 (0.3)	31.4 (0.0)	8.3 (0.0)
Mid	70	4	6.1 (0.0)	103.1 (0.3)	31.4 (0.0)	8.3 (0.0)
Mean	350	-	6.1 (0.0)	105.2 (0.4)	31.4 (0.0)	8.3 (0.0)
Bot	32	Before 1	6.1 (0.0)	99.4 (0.4)	31.5 (0.0)	8.3 (0.0)
Bot	13	1	6.1 (0.0)	98.0 (0.6)	31.4 (0.0)	8.2 (0.0)
Bot	14	2	6.1 (0.0)	96.8 (0.5)	31.4 (0.0)	8.2 (0.0)
Bot	13	3	6.2 (0.0)	96.0 (0.6)	31.4 (0.0)	8.2 (0.0)
Bot	13	After 3	6.1 (0.0)	96.0 (0.6)	31.4 (0.0)	8.2 (0.0)
Mean	85	-	6.1 (0.0)	97.2 (0.5)	31.4 (0.0)	8.2 (0.0)

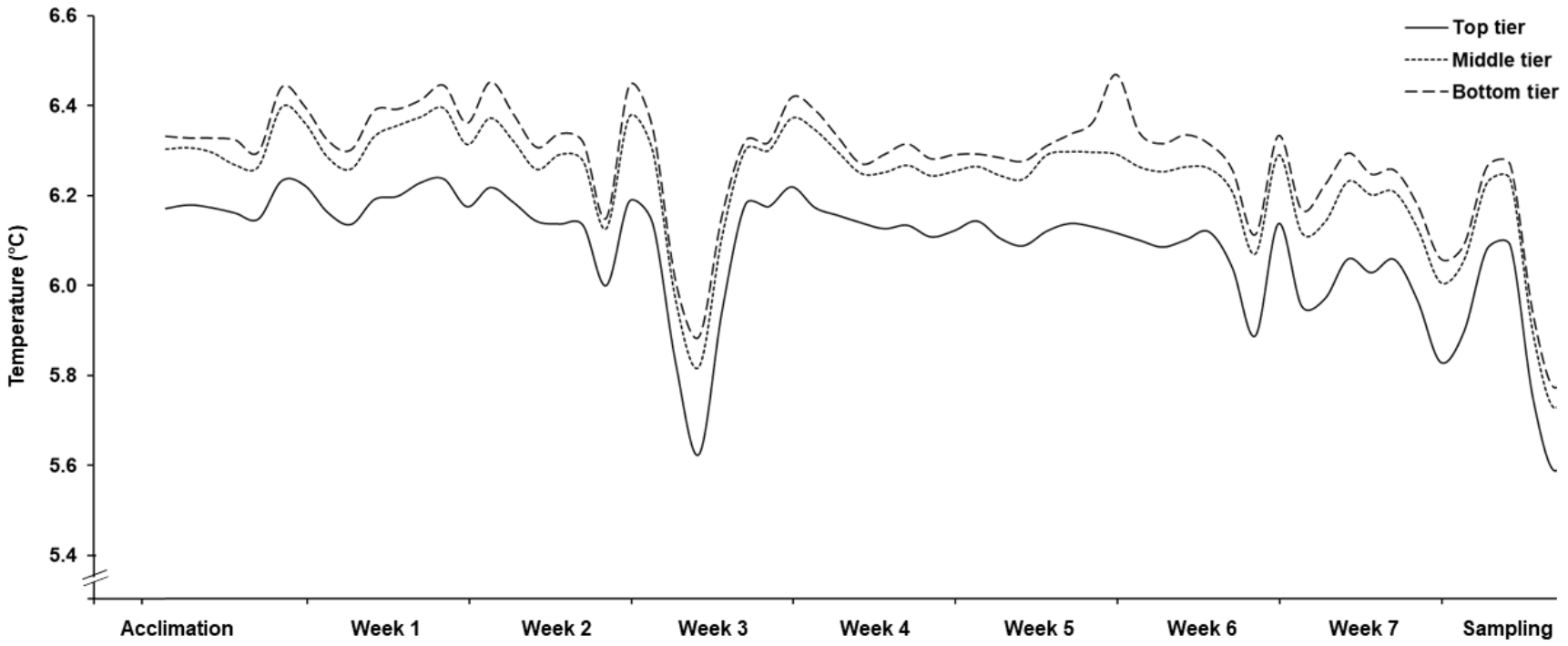

Figure E.1 Daily mean water temperature (ºC) during the last six days of the 10-d acclimation phase, the 7-wk experimental phase, and the 6-d sampling phase of the feed trials, measured every five minutes by two temperature loggers (N=576 each day per tier) placed in the center compartment of the turn in each tier (top, middle, and bottom).

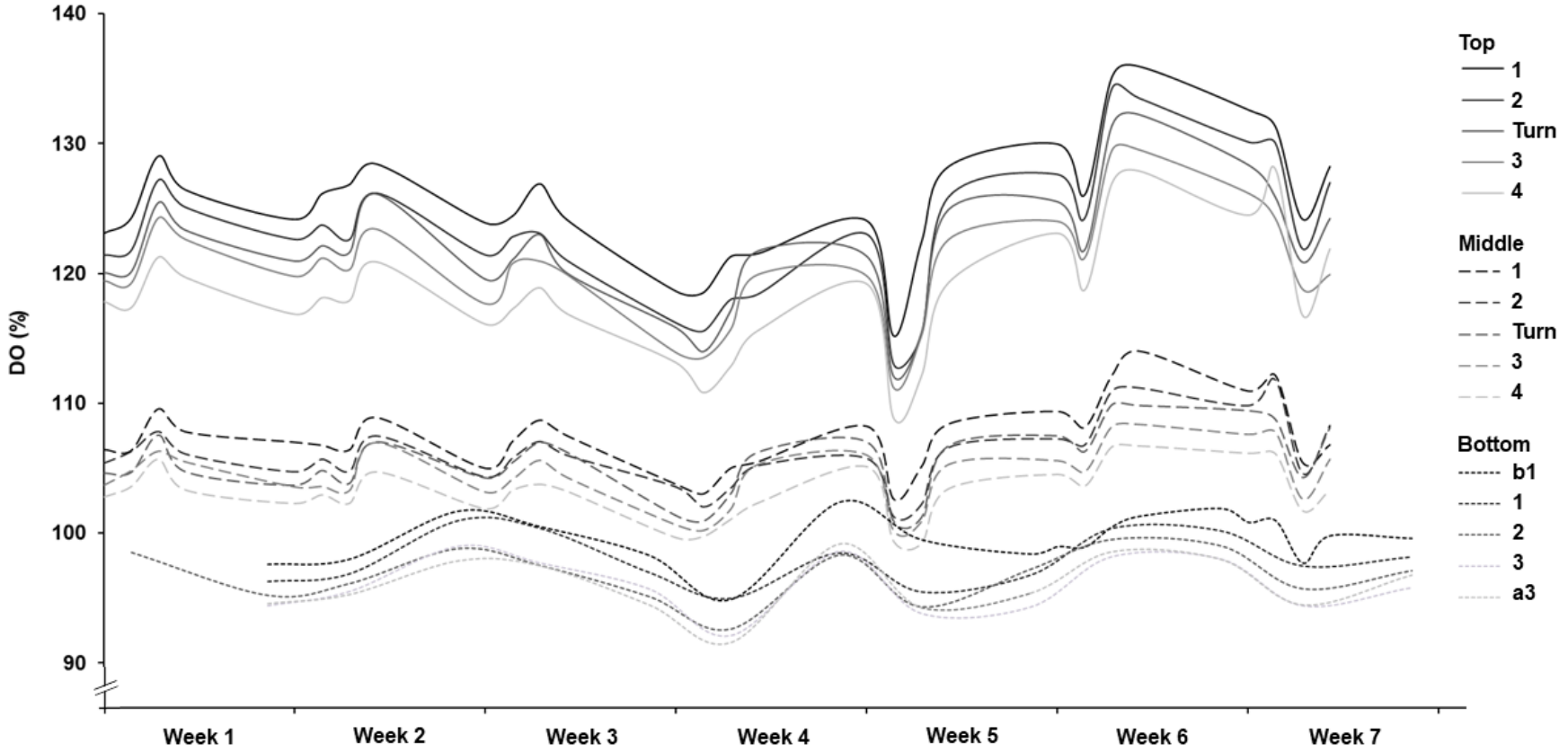

Figure E.2 Daily mean dissolved oxygen (% DO) in the water during the 7-wk experimental phase of the feed trials, measured in the raceway (YSI Professional Plus [Model Pro 10102030]) in the top and middle tiers (positions 1-4 and turn), and in the bottom tier (compartments 1-3, before compartment 1 [b1], and after compartment 3 [a3]). See sections 3.2.4 and 3.2.5 for amount and timing of measures; see Figures 3.2 and 3.3 for tier positions and compartments.

APPENDIX F

Sea urchin aggregation throughout the experiment

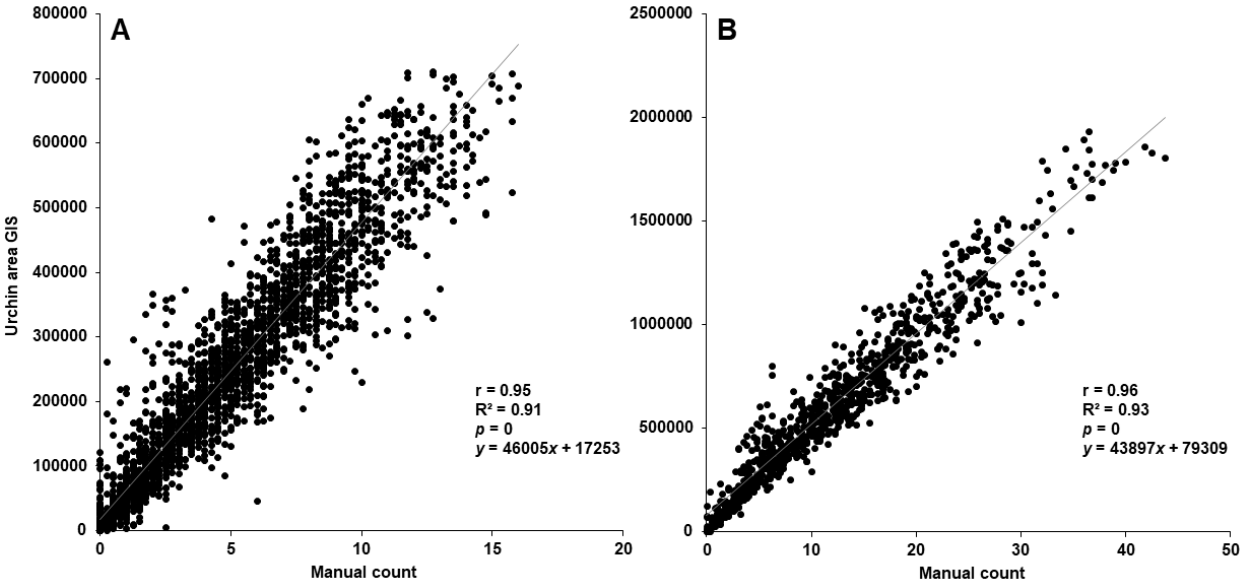

Figure F.1 Relationship (simple linear regression analysis) between the surface area of urchins (determined using the GIS method) and the number of urchins (counted manually), in each of the nine sections of each compartment (A; N=2718) and in each of the three streams (upstream, middle, and downstream) of each compartment (B; N=906) for the 302 sample images. See Figure 3.6 for compartment sections and streams. The strong, highly significant relationships demonstrate that the results from the GIS method are comparable to the manual method, and therefore the GIS results are accurate and acceptable for use in analyses.

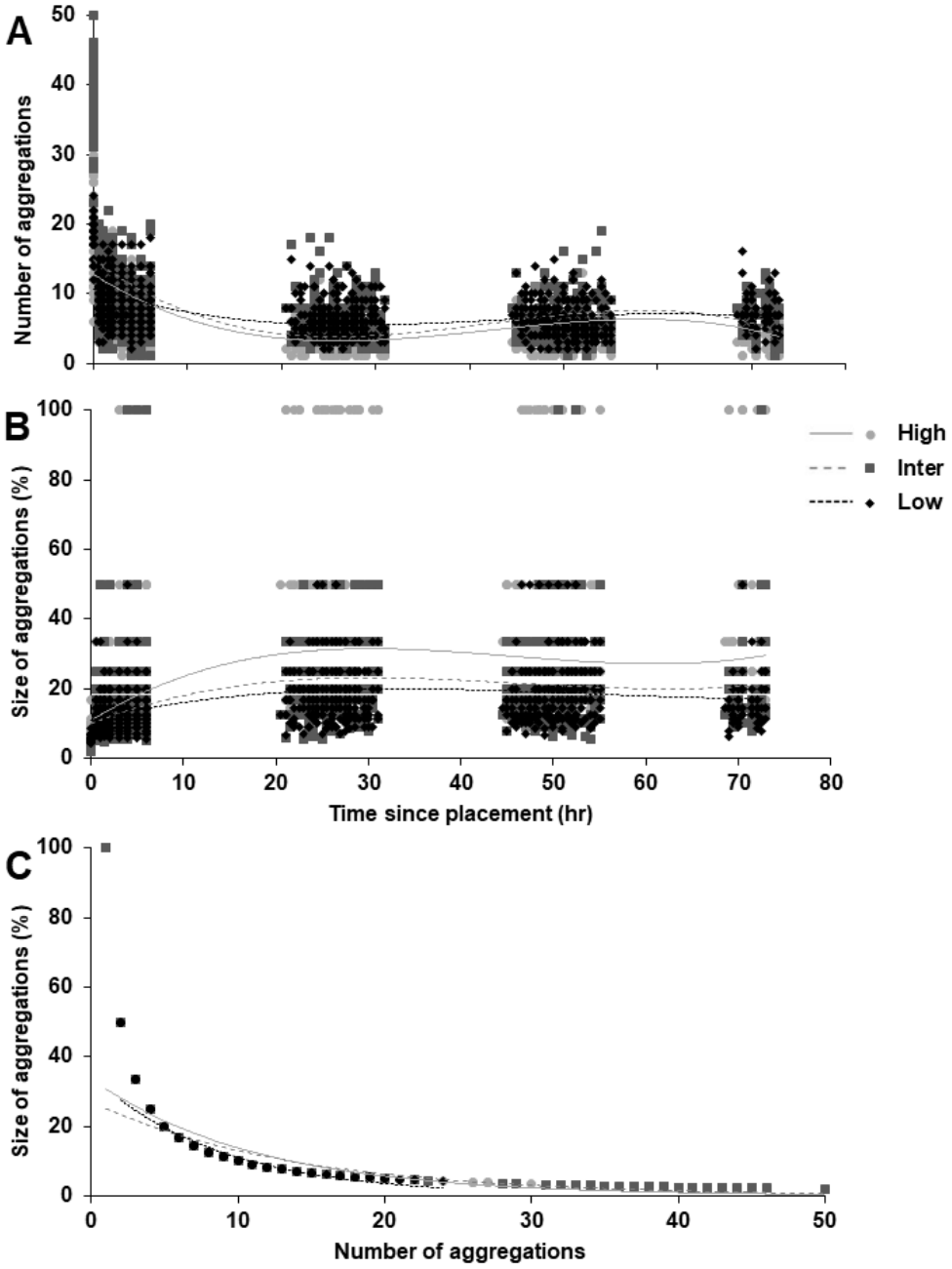

Figure F.2 The change in the number of urchin aggregations (A; N=3021) and the mean percentage of urchins per aggregation (B; N=3021) over time since initial placement of the urchins, and the correlation between the number of urchin aggregations and the mean percentage of urchin per aggregation (C; N=3021), for each of the three urchin stocking densities (low, intermediate [inter], and high; N=1007 per density).

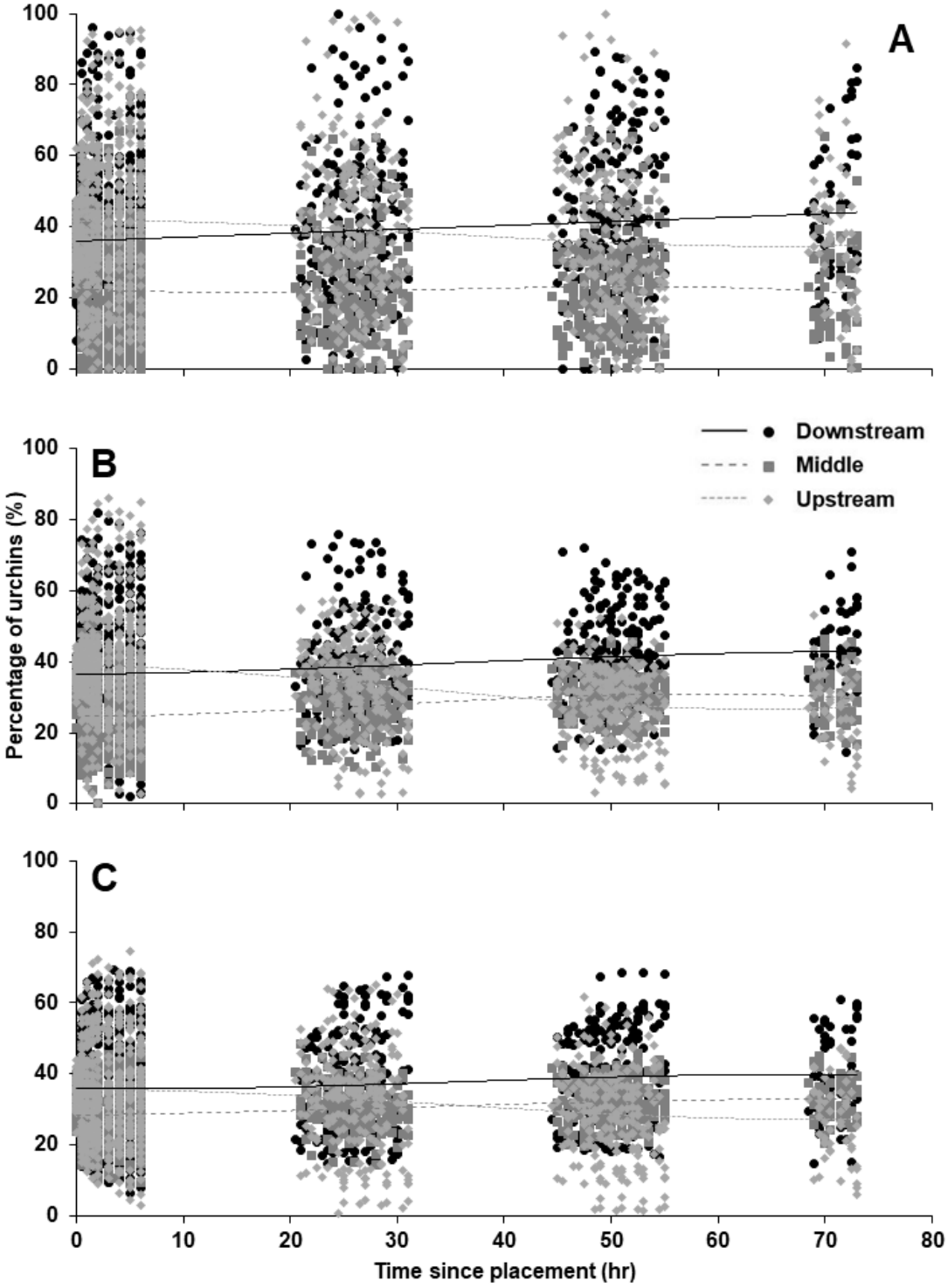

Figure F.3 Percentage of urchins found in the downstream, middle, and upstream areas of each compartment over time since initial placement of the urchins in the low (A), intermediate (B), and high (C) urchin stocking densities (N=3021 per density).

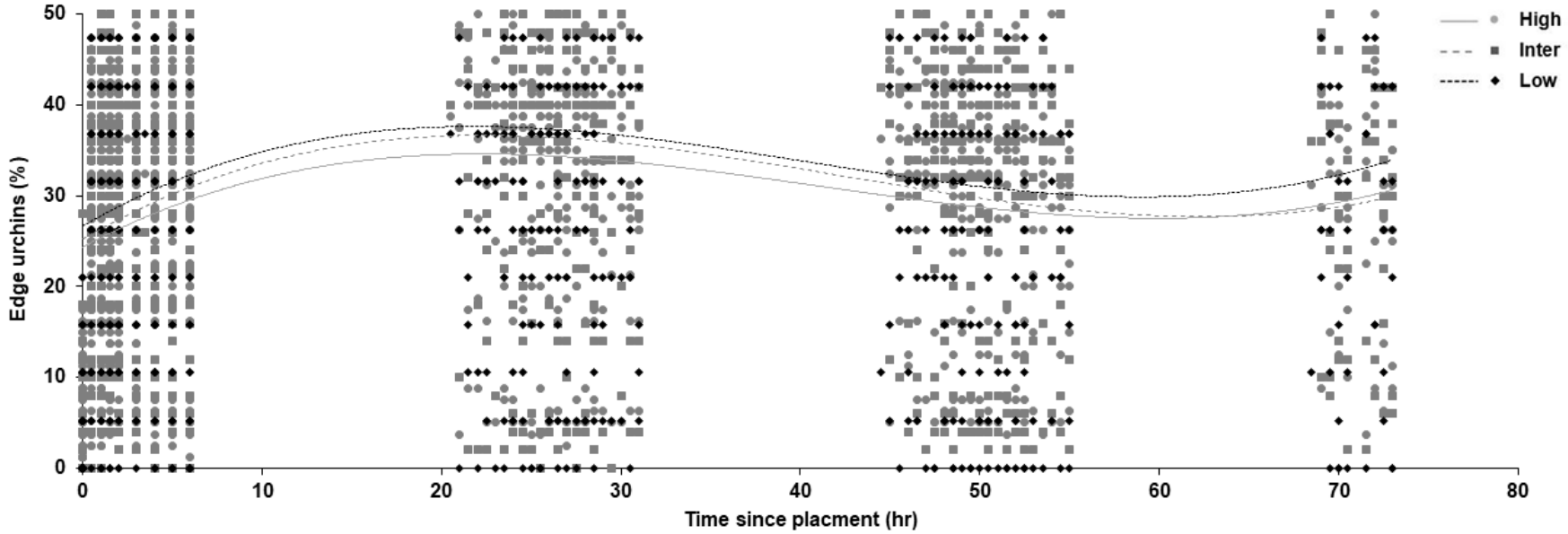

Figure F.4 Percentage of urchins found on the edge of each compartment over time since initial placement of the urchins in the low, intermediate [inter], and high urchin stocking densities (N=1007 per density).

APPENDIX G

Summary of sea urchin bodily characteristics and feed consumption throughout the experiment

Table G.1 Mean (±SE) test diameter, body wet weight, gonad wet weight, gonadosomatic index (GSI), and change in GSI (ΔGSI) of green sea urchins sampled from the barrens (N=50) and kelp bed (N=50) from BCC at the onset and end of the experiment, and from the straight segments of the top two (feed) tiers (N=80 per stocking density; low, intermediate [inter], and high), the turns of the top two (feed) tiers (N=60), and the bottom (kelp) tier (N=139) of the raceway, at the end of the experiment.

Urchin grouping		Test diameter (mm)	Body wet weight (g)	Gonad wet weight (g)	GSI (%)	Δ GSI
Barrens	Onset	47.7 (0.6)	49.9 (1.8)	1.8 (0.1)	3.6 (0.3)	-
	End	48.7 (0.6)	57.1 (1.9)	4.8 (0.4)	8.2 (0.6)	127.1 (16.5)
Kelp bed	Onset	48.4 (0.6)	55.6 (1.9)	9.1 (0.5)	16.5 (0.8)	-
	End	49.8 (0.6)	61.2 (2.2)	6.3 (0.6)	10.2 (0.8)	-37.9 (4.8)
Straight segments (feed)	Total	48.1 (0.3)	54.5 (0.9)	11.3 (0.2)	20.8 (0.2)	474.4 (6.0)
	Low	47.9 (0.4)	53.7 (1.4)	11.4 (0.3)	21.3 (0.4)	489.5 (9.8)
	Inter	47.8 (0.5)	53.8 (1.5)	11.2 (0.4)	20.7 (0.4)	471.3 (11.3)
	High	48.5 (0.5)	56.0 (1.6)	11.5 (0.4)	20.3 (0.4)	462.3 (9.9)
Turns (feed)		48.8 (0.6)	57.6 (2.0)	11.6 (0.5)	20.2 (0.5)	458.1 (13.2)
Bottom tier (kelp)		49.3 (0.4)	59.1 (1.3)	6.9 (0.2)	12.0 (0.3)	231.1 (8.3)

Table G.2 Mean (±SE) relative feed consumption (RFC; g feed kg^{-1} urchin day^{-1}) for the straight segments (N=24 per trial) and the turns (N=6 per trial) in the top two (feed) tiers of the raceway, during each trial (1-7).

Trial	Straight segments	Turns
1	5.7 (0.1)	6.9 (0.2)
2	6.0 (0.2)	6.2 (0.2)
3	6.8 (0.1)	6.9 (0.2)
4	7.6 (0.1)	8.2 (0.5)
5	7.6 (0.1)	8.2 (0.2)
6	7.4 (0.2)	7.9 (0.2)
7	6.6 (0.1)	7.1 (0.2)
Mean	6.8 (0.1)	7.3 (0.1)

APPENDIX H

Summary of full model ANOVAs

Table H.1 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), position (P; positions 1-4 in each tier), and covariate trial (Tr; trials 1-7) on relative feed consumption (RFC) of green sea urchins (*Strongylocentrotus droebachiensis*) in the raceway (N=168; see details of statistical analysis in section 3.2.7.1).

Source of variation	DF	*MS*	*F*-value	*p*
D	2	0.60	0.88	0.42
T	1	4.38	6.46	0.01
P	3	3.88	5.73	<0.01
Tr	1	32.58	48.07	<0.001
D×T	2	0.91	1.35	0.26
D×P	6	0.28	0.41	0.87
T×P	3	6.02	8.88	<0.001
D×Tr	2	0.16	0.23	0.79
T×Tr	1	0.26	0.38	0.54
P×Tr	3	0.65	0.96	0.41
D×T×P	6	0.41	0.61	0.72
D×T×Tr	2	0.04	0.07	0.94
D×P×Tr	6	0.54	0.80	0.57
T×P×Tr	3	0.29	0.42	0.74
D×T×P×Tr	6	0.36	0.53	0.78
Residuals	120	0.68		
Corrected total	167			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.2 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), and position (P; positions 1-4 in each tier) on the mean number of aggregations of green sea urchins (*Strongylocentrotus droebachiensis*) in each compartment after 20 hours from initial placement (N=168), controlling for the random factor trial (1-7; see details of statistical analysis in section 3.2.7.2).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
T	1	0.74	0.22	0.64
P	3	43.18	13.22	<0.001
D	2	92.54	28.33	<0.001
T×P	3	3.31	1.01	0.39
T×D	2	7.13	2.18	0.12
P×D	6	4.40	1.35	0.24
T×P×D	6	3.38	1.03	0.41
Error	144			
Corrected total	167			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.3 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), and position (P; positions 1-4 in each tier) on the mean percentage of green sea urchins (*Strongylocentrotus droebachiensis*) in each aggregation of each compartment after 20 hours from initial placement (N=168), controlling for the random factor trial (1-7; see details of statistical analysis in section 3.2.7.2).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
T	1	0.0	0.00	0.99
P	3	1629.8	13.27	<0.001
D	2	5537.6	45.10	<0.001
T×P	3	155.1	1.26	0.29
T×D	2	52.4	0.43	0.65
P×D	6	437.7	3.56	<0.01
T×P×D	6	93.8	0.76	0.60
Error	144			
Corrected total	167			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.4 Summary of five-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), position (P; positions 1-4 in each tier), and area within each compartment (A; downstream, middle, and upstream) on the mean percentage of green sea urchins (*Strongylocentrotus droebachiensis*) found in each compartment area after 20 hours from initial placement (N=504), controlling for the random factor trial (1-7; see details of statistical analysis in section 3.2.7.2).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
T	1	0.0	0.00	1.00
P	3	0.0	0.00	1.00
D	2	0.0	0.00	1.00
A	2	5803.6	43.89	<0.001
T×P	3	0.0	0.00	1.00
T×D	2	0.0	0.00	1.00
P×D	6	0.0	0.00	1.00
T×A	2	1071.0	8.10	<0.001
P×A	6	2805.0	21.21	<0.001
D×A	4	998.5	7.55	<0.001
T×P×D	6	0.0	0.00	1.00
T×P×A	6	425.9	3.22	<0.01
T×D×A	4	347.9	2.63	0.03
P×D×A	12	251.0	1.90	0.03
T×P×D×A	12	178.7	1.35	0.19
Error	432			
Corrected total	503			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.5 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), and position (P; positions 1-4 in each tier) on the mean percentage of green sea urchins (*Strongylocentrotus droebachiensis*) on the edge of each compartment after 20 hours from initial placement (N=168), controlling for the random factor trial (1-7; see details of statistical analysis in section 3.2.7.2).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
T	1	10.99	0.31	0.58
P	3	930.32	26.39	<0.001
D	2	230.29	6.53	<0.01
T×P	3	65.24	1.85	0.14
T×D	2	102.33	2.90	0.06
P×D	6	57.51	1.63	0.14
T×P×D	6	80.81	2.29	0.04
Error	144			
Corrected total	167			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.6 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), and location (L; 1 and 2 in each tier) on mean gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the raceway (N=24), controlling for the random factor block (1: North compartments and 2: South compartments; see details of statistical analysis in section 3.2.7.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
D	2	2.01	1.17	0.35
T	1	3.64	2.12	0.17
L	1	2.99	1.75	0.21
D×T	2	0.21	0.12	0.89
D×L	2	0.04	0.02	0.98
T×L	1	0.33	0.20	0.67
D×T×L	2	1.70	0.99	0.40
Error	12			
Corrected total	23			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.7 Summary of four-way ANOVA (applied to raw data) examining the effect of urchin stocking density (D; low, intermediate, and high), tier (T; top and middle tiers), and location (L; 1 and 2 in each tier) on the change in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the raceway (N=24), controlling for the random factor block (1: North compartments and 2: South compartments; see details of statistical analysis in section 3.2.7.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
D	2	2.01	1.17	0.35
T	1	3.64	2.12	0.17
L	1	2.99	1.75	0.21
D×T	2	0.21	0.12	0.89
D×L	2	0.04	0.02	0.98
T×L	1	0.33	0.20	0.67
D×T×L	2	1.70	0.99	0.40
Error	12			
Corrected total	23			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.8 Summary of one-way ANOVA (applied to raw data) examining the effect of urchin grouping (G; barrens onset and end, kelp bed onset and end, straight segments [feed], turns [feed], and bottom tier [kelp]) on the gonadosomatic index (GSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the raceway (N=639; see details of statistical analysis in section 3.2.7.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
G	6	3469.2	239.0	<0.001
Error	632	14.5		
Corrected total	638			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value

Table H.9 Summary of one-way ANOVA (applied to raw data) examining the effect of urchin grouping (G; barrens end, kelp bed end, straight segments [feed], turns [feed], and bottom tier [kelp]) on the change in gonadosomatic index (ΔGSI) of green sea urchins (*Strongylocentrotus droebachiensis*) in the raceway (N=539; see details of statistical analysis in section 3.2.7.3).

Source of variation	**DF**	***MS***	***F*-ratio**	***p***
G	4	4034126	458.9	<0.001
Error	534	8790		
Corrected total	538			

DF = degrees of freedom; *MS* = mean squares; *p* = p-value